

North Central Regional Plant Introduction Station

AMES, IOWA













































NCRPIS ANNUAL REPORT – 2019

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NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION NC7 ANNUAL REPORT, JANUARY 1 - DECEMBER 31, 2019

I. PROJECT TITLE:

NC7 "Plant Germplasm and Information Management and Utilization"

II. COOPERATING AGENCIES AND PRINCIPAL LEADERS (current):

A. Administrative Advisor

*J. Colletti, Interim Dean, Iowa State University, CALS

B. Regional Coordinator

*C. Gardner, USDA-ARS, Iowa

C. State Experiment Stations Representatives

Voting members:

1. Illinois	E. Sacks	7. Missouri	S. Flint-Garcia
2. Indiana	L. Hoagland	8. Nebraska	D. Santra
3. Iowa	T. Lübberstedt	9. N. Dakota	B. Johnson
4. Kansas	M. Stamm	10. Ohio	P. Jourdan
5. Michigan	A. Iezzoni	11. S. Dakota	M. Caffe-Treml
6. Minnesota	A. Lorenz	12. Wisconsin	W. Tracy

Non-voting participants:

Non-voting participants.				
13. California-Davis	R. Karban	30. Missouri	S. Flint-Garcia	
14. Connecticut	M. Brand	31. Missouri	S. Jose	
15. Delaware	R. Wisser	32. Nebraska	C. Urea	
16. Hawaii	G. Presting	33. New Jersey	S. Handel	
17. Illinois	J. Juvick	34. New York	J. Doyle	
18. Illinois	G. Kling	35. New York	M. Gore	
19. Illinois	S. Korban	36. New York	P. Griffiths	
20. Illinois	D. Lee	37. New York	A. Hastings	
21. Indiana	J. Janick	38. New York	M. Smith	
22. Iowa	K. Lamkey	39. Oregon	A. Liston	
23. Kansas	A. Fritz	40. South Dakota	L. Xu	
24. Kansas	W. Schapaugh	41. Wisconsin	H. Kaeppler	
25. Kansas	M. Jugulam	42. Wisconsin	S. Kaeppler	
26. Kentucky	T. Phillips	43. Wisconsin	N. de Leon	
27. Michigan	R. Grumet	44. Texas	D. Baltensperger	
28. Michigan	J. Hancock	45. Texas	N. Subramanian	
29. Mississippi	S. Popescu			

1. ARS National Program Staff, Plant Germplasm	*P. Bretting
2. ARS Plant Exchange Office	*G. Kinard
3. ARS Area Director, Midwest Area	*J.L. Willett
4. Cooperative State Research, Education and Extension Service	
5. National Center for Agric. Util. Research	*T. Isbell
6. National Laboratory for Genetic Resources Preservation	*S. Greene

E. North Central Regional Plant Introduction Station, Ames, Iowa

See organizational chart, Figure 1 in the Appendix.

III. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Personnel changes (June, 2019 – May, 2020):

<u>Departures</u>:

• Kurt Kabriel, ORISE staff, IT Support, May 30, 2020

Promotions:

- Brady North, USDA-ARS Agri. Research Science Tech. (Maize Curation), (grade)
- Kallie Judson, USDA-ARS Agri. Research Science Tech. (Entomology), (grade)

New Hires:

- Ashley Sonner, USDA-ARS PFT Biological Science Lab Tech. (previously a TPT employee) (Seed Storage), November 2019
- Dr. Adam Vanous, USDA-ARS TFT Cat 3 Support Scientist (GEM), March 2020
- Adam Spencer, USDA-ARS PFT Secretary Office Automation, March 2020

Vacant USDA-ARS Positions:

Agri. Research Science Tech. (Horticulture) – to be filled June 21, 2020

Vacant ISU Positions: None

Appendix Figure 1 illustrates the organization of the NCRPIS staff and their roles.

Management of Federal and ISU Student Temporary Employees:

USDA-ARS resources provided for 19 student FTE (full-time equivalent) part-time temporary positions in FY 2019, primarily via the Research Support Agreement with Iowa State University. The ORISE and other temporary positions support curatorial activities including regeneration, seed processing, viability testing, farm and facilities operations, IT support, and the GEM Project. Students were interviewed and selected by ISU Program Manager Fred Engstrom. Marci Bushman, PIRU Program Support Assistant, managed the administrative aspects of all student hires, with support and guidance from Admin. Officer Candace Weuve and Program Support Assistant Orlando Guzman.

Budget:

We appreciate the support of the Agricultural Experiment Stations of the North Central Region, which have maintained their annual support and continued to provide \$522,980 in Hatch funds. These funds support the salaries of our nine ISU staff members, their professional travel, and some expenses. In addition, Iowa State University's Agricultural Experiment Station provides support valued at over \$400,000 annually that supports infrastructure, administration, and benefits for current NCRPIS-ISU staff members and retirees.

We are grateful that Hatch funding resources were maintained throughout the difficult sequestration period, and hope they continue to be stable or increase in the future. Currently, about 96% of Hatch NC7 funds are devoted to the wages and salaries of the nine permanent ISU employees. In the near future we will be unable to provide incremental salary increases due to Hatch funding constraints. ISU wage increases will be 2% in 2019, and this figure climbs to 97% if an additional 1% increase

is granted in 2020, barring personnel changes. This limits professional meeting travel, technical training, and temporary student hiring with ISU resources.

FY2019 USDA-ARS funding was essentially the same as final FY2014 funding, minus a one percent assessment for 'Big Data' and smaller assessments for Digitop and SAS licenses. The PI CRIS was funded at \$2.38M (net to location) and the GEM CRIS at \$1.32M. Student hiring for summer 2019 was challenging, despite raising our starting wage for ISU students by almost \$2/hour to \$12/hour, and we were not quite able to fulfill the need for 25 summer FTE, but much better than in 2017 or 2018. We attribute this to both the requirement for all agriculture students to complete internships, and the growing disparity in what we can offer for wages versus other hiring opportunities. ISU Program Manager Fred Engstrom advertised positions more widely across ISU colleges and excellent students were employed from diverse academic backgrounds seeking a hands-on experience with plants. Their diversity of skills was put to good use.

Any reductions in funding will force reduction in student hiring, necessary for executing our genebank's mission. Like many other research units, our ability to cover all aspects of our mission is challenged. Our personnel strive to cover all functions and serve the collections entrusted to us and our stakeholders to the best of our ability. Given the high turnover since 2014, a great deal of time and attention has been paid to recruitment and hiring activities. We feel very fortunate to have filled three more ARS positions supporting Seed Storage, GEM (Germplasm Enhancement of Maize) and office administration with excellent candidates. The research technician supporting Horticultural crop curation is to start in June 2020. A new position to replace the research leader who plans to retire will be recruited in 2020.

Construction and Facilities:

Seed storage space is limiting and needs to be addressed within the next three to four years. The 2018 request to ARS leadership for support for a 2500 sq ft -20°C cold storage building to support essentially doubling the longevity of viability of many of our taxa was added to the Agency Construction Plan, and funds provided in FY2019 for design. A contractor was hired to work with station staff on design and location considerations. In general, space is extremely tight for all personnel and functions. Addition of this building will enable splitting the collection inventories appropriately between 4°C and -20°C, greatly extending longevity of viability.

Greenhouse pest control continues to be augmented with biological controls such as green lacewings (*Chrysoperla rufilabris*), ladybugs (*Hippodamia convergens*), a whitefly parasite (*Encarsia formosa*), and rove beetles (Staphylinidae, *Dalotia*) to help control thrips and other insects.

Please see the Information Management section of this report for details on upgrades that continue to enhance the NCRPIS' information technology infrastructure, and the Farm Support Team section for updates on maintenance and equipment.

IV. PROGRESS IN GERMPLASM AND INFORMATION MANAGEMENT, RESEARCH, AND EDUCATION (C. GARDNER):

(Part IV. summarizes the accomplishments and progress for calendar year 2019, presented in greater detail in the individual staff reports in the document.)

Technical Exchange: An exchange relationship continues to expand between the NCRPIS maize genebank and the CIMMYT maize genebank in Mexico. In addition to regeneration of highland tropical maize at CIMMYT's Tolucca site, new curatorial and database personnel plan to spend time with Ames staff when travel is again possible, post-pandemic. Maize accession information resources and management tools are compared and augmented by both groups.

Acquisition and Documentation Highlights:

In 2019, collection development continued with the acquisition of 293 new accessions (Appendix Table 1). Details are provided in the individual curators' report sections. A historical perspective to provide comparison of acquisitions over the past nine years is provided below.

Year	# New Accessions
2019	437
2018	293
2017	250

Year	# New Accessions
2016	786
2015	229
2014	766

Yea	.r	# New Accessions
201	3	192
201	2	470
201	1	485

The U.S. is now a partner to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Implementation by the NPGS is still under development as several Departments are involved. International collection continues to be challenging as countries adopt variations of the SMTA or other requirements that the NPGS cannot accept. Of ongoing concern is the successful entry of germplasm collected from international explorations into the U.S. It is critical that clean, pest-and pathogen-free seed be shipped or carried in by collectors; sufficient time needs to be devoted to collection sample preparation and sufficient care post-collection. Excellent quantities of seed provided by collectors of many new accessions have made a significant proportion available and distributable immediately.

Original seed samples continue to be scanned in order to provide useful visual references for comparison of regeneration lots with original samples.

Regeneration and Maintenance Highlights:

Regeneration was attempted for 1,562 accessions. Of these, 1,201 were harvested (Appendix Table 2); efforts are described in the curators' report sections. Contrasts with previous regeneration attempts are provided in the table below. Differences may reflect resource levels available for maintenance efforts, challenges due to available hiring authorities and/or labor supply variability over the past decade. Overall collection availability is 77%, an increase of 1% over 2018, despite 8% growth in collection size since 2006.

Year	# Accessions Regenerated
2019	1562
2018	1245
2017	1601

	# Accessions
Year	Regenerated
2016	1033
2015	1627
2014	1230

Year	# Accessions Regenerated
2013	1148
2012	759
2011	1069

A 1,000 row tropical maize winter nursery planted in fall 2019 near Puerto Vallarta, Mexico was deemed successful with high quality seed return in early 2020. Continuing positive results with this nursery provider and our experience with procurement personnel for this effort encourage us to continue to invest in these efforts to make maize germplasm available. Maize germplasm from Thailand was grown under quarantine permit a second year in a winter greenhouse on the Iowa State University campus. Plant growth results were disappointing, likely due to a combination of light and temperature resources.

Assistance in regeneration was provided by USDA-ARS staff of Parlier, CA for increase of wild *Helianthus* taxa. *Daucus* regeneration efforts were supported by seed increases from Seminis Vegetable Seeds (L. Maupin) and from Bejo Seeds (R. Maxwell). USDA-ARS in Salinas, California (B. Mou) supported seed increase of domesticated spinach, and USDA-ARS in St. Croix supported increase of 21 tropical maize inbreds and populations.

USDA-ARS staff of Mayaguez, PR (R. Goenaga) and the St. Croix quarantine nursery staff supported regeneration of tropical maize accessions. Raleigh ARS GEM Project Coordinator Matt Krakowsky provided increases of GEM lines and the Ames GEM team regenerated and provided GEM lines. CIMMYT, the International Center for Maize and Wheat Improvement, regenerated highland maize, which has very specific growing requirements, at their Tolucca site.

Spinach regenerations continue to be supported by cooperative efforts between the USDA-ARS and Sakata Seed America, Inc. in Salinas, CA.

Accessions backed up at the National Laboratory for Genetic Resource Preservation (NLGRP) in Ft. Collins in 2019 numbered 623, with an overall collection backup average of 80%. There is wide variation for percent backup across the various crop collections, from 10% (teosinte, a maize ancestor) to 100% for flax (Appendix Table 2). Variation may be due to lack of appropriate environmental conditions to support the growth and reproductive requirements for some taxa, lack of methods to induce and synchronize flowering for some, and/or insufficient representation of male/female individuals, among other factors. No accessions were sent to Ft. Collins for inclusion in the 2019 NPGS deposit to the Svalbard Global Seed Vault.

	# Accessions
Year	Backed Up
2019	623
2018	795
2017	595

	# Accessions
Year	Backed Up
2016	428
2015	431
2014	1231

	# Accessions
Year	Backed Up
2013	781
2012	799
2011	792

Distributions:

Approximately 33% of the 2019 germplasm distributions were to international and 67% to domestic requestors. Distributions continued to reflect high demand in 2019 (Appendix Table 3). The 2017-2019 timeframe reflects 30-50% higher distributions than the previous three years.

Year	# Items	# Unique Accessions	# Orders	# Requestors
2019	54,232	22,271	1,296	902
2018	61,124	23,229	1,414	1,000
2017	55,474	22,801	1,410	1,019
2016	39,520	18,093	1,254	963
2015	34,188	14,279	1,186	945
2014	41,655	17,558	1,285	993
2013	40,409	17,788	1,523	1,204
2012	45,115	18,811	1,632	1,344
2011	38,402	18,634	1,501	1,180
2010	26,651	13,226	1,183	820
2009	26,904	13,515	1,487	1,081

Non-research requests (home gardeners), continue to heavily target vegetable and ornamental germplasm, more than one-half of all orders to NC7 are cancelled non-research requests; other NPGS sites are also heavily targeted. Home gardeners are redirected to other sources of commercially available materials. Although our resources cannot support maintaining and distributing the collections to home gardeners, we inform these requestors about plant genetic resource conservation and encourage interested individuals to save seeds, conserve them, and share germplasm and associated information. The proliferation of websites instructing non-research requestors how to deceive curators at various germplasm sites in order to get free germplasm continues to be problematic. The careful efforts that go into each and every increase, characterization, imaging, processing, storage, viability testing, and distribution surely make these seeds among the most expensive to provide. GRIN-Global's user friendly order module also helps individuals to select more diverse germplasm from a number of NPGS sites at once.

The relative numbers of distributions generally correlate well with the proportional makeup of the collections and vary from year to year, although demand for maize is usually greater than for other crops.

Curator	Collection Size 2018		% of 2019 Distributions	% of 2018 Distributions	% of 2014 Distributions
Brenner	9,272	17	21	13	14
Carstens†	3,772	7	1	1	<1
Marek	12,775	23	32	27	16
Millard/ Bernau†	21,097	38	32	43	35
Reitsma	7,858	15	14	16	28
Totals	54,774	100	100	100	100

†Barney collections assumed by Carstens in late 2015; Bernau became our 2nd maize curator in 2019.

Research demand for our plant genetic resource collections continues to be very high; requests for diversity and relationship analyses, disease and pest resistance, biofuel, and health and nutrition contribute to these increases, as well as for basic research applications such as photoperiod response, and an array of performance traits. There was unusually high demand in 2017-2019 for almost all major crop species. Germplasm requests continue to be driven by publication of information from genomic (genotyping by sequencing) and phenotypic analyses projects, some or the studies are supported with SCRI, AFRI, or NSF funding.

Evaluation and Characterization:

With the enhancements made to the 'Attachment Wizard' that work in conjunction with GRIN-Global, image loading has resumed. A large volume of accession-associated images and other types of documents is being attached to accessions, orders, and inventories. In 2019, more than 5200 images were loaded and associated with about 2600 accessions. More than 26,500 trait observations were loaded/associated with 2,764 accessions. For the NC7 collections, 41,938 accessions have one or more trait observation data points available via the GRIN-Global database, (https://www.ars.grin.gov/npgs/).

In 2019, the NCRPIS utilized more than 6,000 accession items internally for germination, seed increase, observation, evaluation and characterization for a wide array of descriptor information, and for viability testing (Appendix Tables 2, 4). Other uses include pathogen testing to meet international distribution requirements and back up and herbarium preparation.

Bayer Crop Science provided in-kind support, screening maize inbred accessions for Goss' wilt resistance in Nebraska and for tar spot resistance in Illinois. Corteva Agriscience provided in-kind screening of maize inbreds for fusarium resistance and for head smut resistance.

Information technology and telecommunications:

The NCRPIS staff continues to provide expertise and leadership for the development of GRIN-Global (GG), the successor to the GRIN system, implemented in 2015. This has been the sole primary focus of NCRPIS developer Pete Cyr since 2008, and a major focus of two other NCRPIS staff members, Mark Millard (system analyst) and Lisa Burke (Advisory Committee Chair, beta testing, training) with substantial time invested by additional personnel. The Database Management Unit (DBMU) in Beltsville, MD is responsible for hosting and maintaining the database and the system, developing the public interface, GRIN Taxonomy, changes to the system's Middle (business) Tier and administration. Periodic video training conferences continue to be offered by DBMU personnel (contract documentation specialist Marty Reisinger) for NPGS site personnel training, as for the past five years, and other training as requested.

Software development efforts continue to center on the development and deployment of user tools that improve curatorial workflows, user experience, applications for data capture and transfer, enabling increased availability of accession-associated information to the public. These efforts are facilitated by contributions from germplasm stakeholders in the U.S. and abroad, as we seek examples of use cases and

desired features and functionalities of the new system. A formal process is used to submit and address enhancement requests, prioritize development, assign work to developers, and to securely share new software applications between GG adopters to extend the system's functions and features.

Sixteen national or international genebanks have now implemented GRIN-Global for genebank use, and many of these have live public interfaces. Another 25 are in the process of evaluating and/or implementing the system, truly evidence of global adoption of this valuable resource.

For almost five years, the NPGS has utilized a GRIN-Global Advisory Committee (AdCom) as a forum for genebank personnel and developers to identify development needs, prioritize them, test, and approve software for release. The AdCom is chaired by NCRPIS staff member Lisa Burke and has been highly productive. An international AdCom was formulated with participation by key personnel from the Crop Trust, the US NPGS, CIMMYT, and CIP, and confers monthly. A process was developed for international development products to be checked into branches of the Git vault (maintained by the Trust at CIMMYT) and then vetted.

One focus of our current ARS Program cycle is to develop inter-operability between GG and other key information providers' portals, examples including MaizeGDB, Gramene, LIS (Legume Information System), or GOBii. Pete Cyr is investigating the use of BrAPi tools, which use a RESTFUL webservice interface, to achieve these objectives.

Please see the IT section for technical details of NCRPIS support activities. We owe IT Specialist Jesse Perrett, ORISE fellow Kurt Kabriel, and Fred Engstrom sincere thanks for implementing the NCRPIS intercom communications system and the Monet environmental monitoring system. The Monet system provides continuous remote monitoring capability for greenhouses, cold storage areas, dryers, and other areas where extreme changes could impact processes or seed quality.

Germplasm's Viability and Health:

In 2019, about 10% of the collection was tested for viability in 2019, more than 5700 accessions, as we have increased resources available for determining collection quality. A concerted effort is being made to assure all seed lots 10 years or older have current germination information. Our storage conditions (4 C, 25-35% relative humidity) are very good, and the efforts devoted to seed cleaning ensure storage of very clean seed lots, important to longevity of viability. Construction of a -18C cold storage building will provide for much longer period of viability for many of our taxa. This would bring significant cost savings over the long term, as most of the collection's seeds lose viability long before inventory supply is depleted. Less frequent regeneration need would enable more rapid progress in making the collection fully available.

ARS Pathologist Dr. Anna Testen has provided each curatorial team with guides and protocols for improved field and greenhouse practices to support healthy plant and propagule production. Collaborations continue for development of methods to

eliminate the bacterial fruit blotch pathogen, *Acidovorax avenae*, from *Cucumis melo* seed.

Field inspections were made for all crops. All cucurbit seedlings were screened routinely for presence of Squash Mosaic Virus via ELISA; Outcomes are detailed in the pathology section of this report.

We continue to test for adventitious presence (AP) of genetically engineered organisms (GEO) in maize germplasm accessions new to the NCRPIS and sampled newly produced seedlots, using a commercial laboratory vendor.

Insect management:

The Entomology staff provided six insect pollinator species to control pollinate 989 accessions. Honeybees continue to be the primary pollinator used in the NCRPIS regeneration program, followed by the Alfalfa Leafcutter Bee (ALC).

Detailed, interesting observations and interpretative information regarding their field pollinator research activities can be found in their extensive section of the annual report for information on their continuing efforts to enhance the pollination program's effectiveness and efficiency. Substantial reporting is devoted to this team's activities because of the uniqueness of this project, limited sources of such information, and relevance to the broader germplasm conservation world. Feedback and suggestions on experimental approaches are welcomed.

Effectiveness of insect pollinators on cross-fertilization of caged plantings, and preservation of the genetic profile of the accessions is considered during regeneration.

Enhancement:

The Germplasm Enhancement of Maize Project (GEM) works with 64 active public and private collaborators to adapt exotic maize germplasm to broaden the genetic diversity of temperate U.S. maize production and provide unique, key priority traits. Research and breeding are designed to improve exotic germplasm introgression methods, to provide unique sources of allelic diversity, and to identify traits and genes to support improvement of agronomic productivity, disease resistance, insect resistance, and value-added grain characteristics of importance to human health and nutrition. International collaborators are screening GEM germplasm for late wilt, tar spot, maize rough dwarf virus, corn stunt, and others.

The Ames and Raleigh, NC GEM Projects and public collaborators have released 332 lines from 2001-2020 representing more than 60 maize races. An important goal is development of a set of inbred lines representative of the diversity inherent to all of the races of maize. In addition to traditional introgression methods, the project has released 204 doubled-haploid (DH) maize lines in partnership with the ISU Doubled Haploid Facility. The next set of DH lines from the allelic diversity project will be released in Summer 2020. These lines have one-quarter exotic, three-quarters temperate background. In 2017 and 2018, the GEM Technical Steering Group's private sector members tested 47 GEM lines from the Ames and Raleigh, NC programs internally on their own proprietary testers. These data enabled calculation of the first general and specific combining ability estimates for GEM germplasm on

company tester lines, important for breeders and researchers to gain insights into effective use of these genetic technologies. A second, similar trial is planned using lines released by the projects since 2016, again by the private sector collaborators.

Photoperiod sensitive tropical maize often does not flower until September in Ames. GEM and maize curatorial teams have continued to collaboratively develop an effective method for photoperiod control in the field. The sunflower project has also used photoperiod control effectively to induce flowering in certain wild sunflower accessions. Photoperiod-control environment capacity on the order of one to three acres would be very useful in maintaining and providing unique genetic resources.

GEM field days are held every September and are well attended by scientists, breeders and graduate students. The field days offer a unique opportunity for more molecular-focused researchers to understand the diversity of the materials available for research, and the activities that support germplasm development.

Outreach and Scholarship:

More than 400 visitors toured the NCRPIS during 2019. Our staff participated in teaching students from grade K to postgraduate level and provided outreach events to civic and other organizations about germplasm conservation and management, and the work done at the NCRPIS. Scientific and technical staff members continue to publish scholarly journal articles, make presentations at scientific meetings, and supervise graduate research programs.

Current and future foci:

Processes involved in regeneration, characterization, and making viable germplasm available are labor intensive. Resources do not allow maintenance and regeneration efforts (including viability testing) to keep pace with demand. We continue to try to improve conservation methods to better use the resources available to us, and to develop labor and resource saving technologies. ARS leadership approved design of a -20°C cold storage building in FY2020 order to extend longevity of seed viability, and we hope to receive resources for construction in FY2021. We continue to evaluate activities that can be reasonably reduced without sacrificing collection health and quality, and to improve efficiency.

Continued emphasis will be placed on communicating with research stakeholders to address development of comprehensive, genetically diverse collections to meet research and development needs. More emphasis has been requested for advanced breeding materials, doubled haploid germplasm, mapping populations, single mother trees, and ephemeral genetic resources derived from NSF, AFRI, or SCRI-funded research.

Climate change is forcing researchers to renew efforts to identify superior forage cultivars as well, and interest has increased in collections of suitable species. A 'gap analysis' process is utilized to examine distribution of crops and their wild relatives; information sources include herbarium records, floras of various countries and ecoregions, predictive analyses bases on GIS layers and habitat information, and scholarly publications that cite plant sources, traits, and performance attributes. Wise selection of targets is important to managing collection growth and effective use of

resources. The horticulturists' report details how collection priorities have been determined, and how gap analyses affect these priorities.

Better characterization information is essential to enable well-targeted use of the collections, especially given the increasing constraints of limited research and conservation resources. Availability of PGR significantly impacts research applications, including taxonomy.

Implementation of new, optical and spectroscopic-based technologies are in process and we hope they will enable us to better understand seed properties and improve the quality of our seed inventories.

Software development efforts will continue to center on the development and deployment of GRIN-Global resources, and on information management tools that can facilitate information transfer from various providers and integrate the information in useful ways for researchers. These efforts are facilitated by contributions from germplasm stakeholders in the U.S. and abroad, as we seek examples of use cases and desired features and functionalities. A formal process is used to submit and address enhancement requests, prioritize development, assign work to developers, and to securely share new software applications between GG adopters to extend the system's functions and features.

V. IMPACTS OF GERMPLASM USE BY NORTH CENTRAL REGIONAL RESEARCHERS:

Impacts of germplasm use by the researchers at the NCR institutions:

A detailed list of examples of germplasm use in research being conducted at NCR institutions was not requested of the RTAC members this year. NC7 Region researchers typically account for nearly half of domestic plant germplasm distributions from the NCRPIS. Requests for germplasm continue to increase for research as well as non-research use. Requests become increasingly better targeted as the quantity and quality of information associated with the collection improves, thus sharing of findings resulting from use of NPGS germplasm, linked with the germplasm's identity and source, is critically important.

The linkage of the GEM Project, the maize curation project, and public and private collaborators throughout the U.S. facilitates the use of exotic maize germplasm by public and private sector maize researchers. This unique partnership offers great potential for diversifying the genetic base of U.S. maize production, the mission of the GEM Project.

Linkages among project participants and with other projects/agencies and contributions of the Regional Technical Advisory Committee:

Linkages are driven primarily by common research interests and objectives and by the heritage of the germplasm material utilized for research and education. All states utilize germplasm provided by the NCRPIS and many of the other 19 NPGS sites; the states have a complex array of collaborative research efforts between their institutions, and with the plant genetic resource curators at the NPGS sites.

The Regional Technical Advisory Committee (RTAC) has provided valuable direction in the following areas:

- Requesting and suggesting organizational structure of information needed to determine project impact and provide accountability. This includes advice on useful formats for analyzing and evaluating the nature of distributions, whom they benefit, and how benefits are realized, which are essential for determining the impact and value of the project.
- Identifying needed improvements to the public GRIN interface.
- Providing input from their respective AES Directors to curators, genebank and other administrators.
- Providing guidance to increase the NCRPIS program's relevance to NCR stakeholders.
- Providing technical expertise, particularly in the areas of diversity assessment and taxonomy.
- Providing added breadth in understanding issues at genebanks beyond the NCRPIS.
- Understanding the challenges faced by public researchers partnering with other public institutions' researchers, both governmental and non-governmental. This has provided useful insights for ARS and NCR administrators to guide programmatic decision-making, as well as operational guidance; this function is key because of its direct impact on the public interest as well as the specific research interests of more directly involved stakeholders.

The technical committee gatherings provide an opportunity for the AES Directors' representatives to learn about and understand strategic issues which impact how their institutions operate and how they can cooperate more effectively to address their mission in today's environment, and then provide this information to their Directors. Among the benefits for the representatives are the opportunity for exposure to research in areas outside their own area of expertise, leading to greater understanding and insights, and the opportunity for service to their institutions, to the NPGS, and to germplasm security.

The 2019 NC7 RTAC meeting was hosted by the University of Illinois and NC7 RTAC member Erik Sacks. Minutes of this meeting can be found on the NIMSS website.

Some of the NC7 RTAC's specific suggestions and contributions include the following: (from the meeting minutes):

- Concurrence of the review and approval of the 2019/2020 NC7 Hatch Project budget by the NCR AES Directors.
- The committee recognize and thank Host Professor Erik Sacks, Associate Dean German Bollero, Department Head Adam Davis, their colleagues who contributed, and especially Emeritus Professor Ted Hymowitz.
- Discussion of the need to train the future plant genetic resources workforce, as 1/3-1/2 of curatorial personnel are retirement eligible. A Higher Education Challenge Grant application is hoped to provide resources to support development of academic and practical training resources.
- RTAC members expressed concern and encouraged NC7 curators to pursue genomic characterization of all collections and to make this information accessible.
- RTAC members encourage development of a web vehicle to automatically request information on findings/data resulting from use of plant genetic resources and facilitate inclusion of such information in the public GG system, thus extending the utility of these resources for further research.
- The Hatch budget is insufficient to sustain the 9 Iowa State University employees in Ames, according to projections for 2021-2022. RTAC members will discuss this with their Hatch Project Advisors, and Deans.
- Summaries of research projects using plant genetic resources by the NC7 RTAC members at their institutions.

VI. SUPPORT TEAM REPORTS:

A. Farm (F. Engstrom, B. Buzzell, C. Hopkins)

We supervised and coordinated daily operations at the NCRPIS farm, including management of all facilities, fields, and greenhouse space. We conducted all pesticide applications in the field and campus greenhouses. We responded to maintenance requests from staff members at the farm and the campus location. We selected, coordinated, and scheduled the student labor force. We coordinated and completed facility construction and upgrades along with safety inspections.

Labor:

During 2019, 67 applications for hourly employment were received and reviewed. There were approximately 50 interviews, resulting in 29 new and 26 returning hourly employees hired. Currently there are 25.2 (FTE) Biological Science Aides working at the NCRPIS.

NCRPIS Farm Crew Personnel:

- Fred Engstrom, (Program Manager II) Joined the staff July 2016.
- Brian Buzzell (ISU Farm Mechanic) joined the staff in May 2002.
- Cole Hopkins (ISU Agricultural Specialist II) joined the staff in September 2016, and assists the vegetable project half-time, and facility operations half-time.

Maintenance projects:

During the past year the farm staff initiated and completed the following projects which enhanced the efficiency and safety of the station operations.

- 1. Installed a split unit air conditioner for improved climate-control in rooms utilized for vernalization of brassicas.
- 2. Installed a split unit air conditioner unit for temperature control of bee overwintering room.
- 3. Continued the use of biological controls for greenhouse pest control.
- 4. Arranged for repair of compressors, HVAC equipment in multiple areas.
- 5. Replaced various light fixtures with energy efficient LED lights in offices and other work areas as needed.
- 6. Spread 195 tons of rock on roadways at the PI Station to maintain and improve access to fields and facility.
- 7. Installed additional Monnit temperature and humidity sensors in facilities to track trends and alert us to abnormal conditions.
- 8. Responded to a catastrophic heating failure at ISU campus greenhouses during the "polar vortex" the beginning of February. Our crew installed and monitored supplemental heating units around the clock for a week while campus facilities repaired the system. Minimal plant loss was experienced.

Purchasing:

Fred Engstrom coordinated purchasing for the NCRPIS farm: this task included gathering and summarizing requests, writing specifications, and obtaining supplies for the farm.

Equipment Purchased:

- 1. An eight-foot power harrow was purchased and utilized for "spot" tillage of cage frame footprints, allowing for minimal soil disturbance of non-crop field space. This allows the alley ways between cages to have grass or other soil structure retaining plants to remain in place throughout the season decreasing compaction, erosion and increasing access.
- 2. A skid loader mount trencher was purchased for modification to improve mechanical trenching for the cage screening process.

Tours:

During 2019 there were more than 500 visitors.

Staff Training:

We conducted Tractor and Utility Vehicle Safety, Worker Right-to-Know and Worker Protection Standard training sessions for the new staff and student employees as well as annual updates for existing staff.

B. <u>Information Technology and Telecommunications (P. Cyr, J. Perrett)</u>

Jesse Perrett served as the first line of support for NCRPIS during 2019. Jesse is supervised by Pete Cyr who is dedicated to the GRIN-Global project. Jesse supervised

Kurt Kabriel, ORISE intern in assisting with IT/IM related tasks. The following list outlines the progress made by the IT team during 2019 at NCRPIS.

Equipment:

As of December 2019, the NCRPIS had 55 desktop and 35 laptop/tablet workstations installed for use by permanent staff members and part-time temporary student help. All station computers are equipped with solid state drives, have at least eight gigabytes of memory, and quad core processors. The centralized functions required by the station were supported by 13 physical servers and around 20 active virtual servers including those used for file storage, intranet, backups, and access security systems and monitoring.

A firewall was maintained order to provide enhanced security as well as increased network performance in line with the 10-gigabit server network infrastructure. Each server rack is protected by a battery backup. In addition, a station generator system will provide power in the event of power grid failures. The generators in conjunction with the individual rack mounted battery backups should limit the possibility of power failure-related server issues.

The station continues to implement virtual servers wherever possible to better utilize existing server capabilities and improve efficiency. Virtual server hosts use solid state drive tiered storage systems utilizing the technology built into Microsoft Windows Server 2019 to enhance storage performance of existing servers at minimal cost.

Decommissioned old large format printer and installed a new printer. In addition, procured new foldable printing media for poster printing to make travelling with posters more feasible.

Deployed five new Getac T800 G2 field tablets for field data acquisition and pollination request tracking. These tablets are rugged Windows devices with outdoor viewable screens and can keep up with modern IT systems requirements.

Upgraded cold room speakers for use with the Visiplex intercom system. The audio for the new speakers is clearer in larger rooms and should help personnel hear notifications even in the back of the south cold storage room.

Installed new wireless access points in the cold rooms. The new access points allow wireless communications to the network while working in the cold rooms, supporting data capture and retrieval workflows.

Decommissioned and upgraded all Microsoft Windows Server 2008 computers before the January 2020 deadline. Also upgraded as many servers as possible to Windows Server 2019 and decommissioned server hardware that was incompatible with the newest versions of Windows Server 2019.

Implemented a combination wireless system to allow environmental sensor placement and data acquisition from the campus greenhouses. The new system was installed without the need for a hardwired ethernet connection using existing ISU wireless networking equipment and complements the sensors used in the greenhouses located on station.

Assisted with configuration and installation of 24 new LED grow lights in the entomology and headhouse greenhouses. The new lights will allow for both data capture and light color and intensity management in addition to cost savings.

Responded to numerous Client Experience Center (CEC) data calls for converting IT systems to a new centrally managed CEC implementation.

Decommissioned old antivirus system and installed a new system which is centrally managed by USDA. The new antivirus system allows much faster department wide response to virus and malware threats.

Ensured all computers were compliant with department installations of Bigfix for patch and software deployment and update monitoring as well as reporting to ARS IT specialists.

Updated GRIN-Global label programs with enhancements and fixes as needed. Worked with germplasm management staff to configure label printing for simplicity and functionality.

Implemented new headset system for duplex real time wireless communication between individuals working on farm equipment. The new communications system protects hearing while passing through emergency and voice sounds in addition to allowing the planter tractor driver to communicate with the people deploying packets.

Multiple new SharePoint lists were created for tracking purchasing requests, maintenance requests and farm spray records. The new lists allow multiple users to add and monitor requests for new supplies and requests simultaneously. Reporting was set up so individuals get updates when changes were made to the lists.

A new Microsoft Server Updates Services virtual server was installed and configured for the station to allow for more control over Windows Updates. The new server allows approval and tracking of update compliance for all Windows computers on site.

Security system cameras are installed around the station to monitor property entrances and outside activities. This includes two new cameras installed this year.

Staff printed over 5000 field-ready wooden stakes. Issues with print quality and print consistency were resolved to ensure minimal misprints.

Software:

All workstations at NCRPIS use Windows 10. Microsoft Office 2013, Microsoft Office 365, Adobe Acrobat Professional DC, Adobe Creative Suite, Pulse Secure, Activclient, Java, BigFix, and the GRIN-Global Curator Tool were installed on systems as necessary. Laptops and tablets were encrypted by bit-locker.

PDQ Inventory and PDQ Deploy were also used for deployment and monitoring of non-Windows software packages such as the GRIN Global curator tool.

Documentation:

Updated station information system security documentation and disaster recovery plans. The new documentation includes information necessary to repair or reconfigure station IT systems in the event of a natural disaster or equipment failure.

Weather station history data was provided via SharePoint to allow users to download current and past weather data including calculated GDU and CHU (heat unit) data. The station uses a SharePoint Server 2016 Intranet site for advanced document management and retention. Umbraco website management tool was used to configure the NCRPIS public webpage on USDA's website and for posting IT support videos and training documents, and information about farm operation, safety, and health to the NCRPIS intranet website (internal use).

Plans for 2020:

- Continue to update documentation for IT systems and services.
- Perform information systems disaster recovery trials with non-IT Specialists assistance.
- Upgrade systems to be ready for major IT consolidation efforts.
- Fully implement the Omnilert system for contacting and updating employees about emergency station closures and situations.
- Continue to replace NCRPIS workstations on an as needed basis (targeting a 3-5 year lifespan for daily use workstations).

GRIN-Global:

GRIN-Global is the product of a joint partnership between the USDA-ARS NPGS, the Global Crop Diversity Trust and Bioversity International to develop a new genebank information management system that it can be deployed on any size computer with a minimum amount of effort and cost. The GRIN-Global system is currently implemented by 14 national or international genebanks and is being evaluated for adoption by additional 27 other genebank entities. GRIN-Global is designed to support an unlimited number of languages (seven languages are currently installed) and could be configured to support four database engines. The complete GRIN-Global system can be installed on a stand-alone desktop computer or in a network server/client configuration.

The USDA-ARS GRIN-Global development team is located primarily in Ames, Iowa (PIRU) and in Beltsville, Maryland (DBMU). Pete Cyr is responsible for the Curator Tool, Search Tool and development of associated wizards. Curator Tool 1.9.9.4 is in beta now and soon to be released. Development of new Cooperator, Order, and Viability wizard versions was recently completed, and they continue to evolve in response to user needs. Mr. Cyr is also working on software to enable GRIN-Global database information to be more readily linked with genomic information from other providers. Mark Millard serves as the business analyst, and Lisa Burke serves as chair of the GRIN-Global Advisory committee.

The DBMU personnel are responsible for the administration of the GRIN-Global database, the Middle Tier and security features, and the public website (PW), https://npgsweb.ars-grin.gov/gringlobal/search.aspx? PW 2.0 is currently under development.

Plans for 2020:

- Enhance the Viability Wizard to meet NLGRP viability testing needs.
- Enhance Curator Tool Reporting support to enable easier development of Crystal Reports and the addition of support for SQL Server Reports.
- Enhance the Curator Tool to leverage modern connectivity technology to replace the legacy SOAP XML communication technology currently being used.
- Enhance the Curator Tool installation process to minimize the need for administrator permissions steps required to make the Curator Tool work with recent Microsoft security changes.
- Enhance the Curator Tool to present a user-friendly interface for managing dataview tabs, Crystal Reports, and Wizards gracefully.

C. <u>Information Management-Germplasm Collections (S. Estrada)</u>

Acquisition:

The North Central Regional Plant Introduction Station (NCRPIS) acquired 1061 (568 active/493 inactive) new accessions in 2019 in the form of 1921 inventory lots (573 active /811 inactive). Of these new accessions, 248 were received from within the National Plant Germplasm System (NPGS) through exploration and transfer (119 from the Seeds of Success program, eight from other sites, and 121 from the NLGRP. Details of specific acquisitions are found in the curators' sections of this report.

As new accessions are recorded in the Germplasm Resources Information Network (GRIN-Global) database, we include as much passport information as possible. Typical passport information would include a source history, cooperator records, collection-site descriptions and geographic coordinates for wild collections, pedigree, secondary identifiers, IPR considerations, and any additional pertinent information provided by the donor. An excel workbook was developed to streamline the assembly of passport data and aid in loading the data to the GRIN-Global database.

Maintenance:

Curatorial assistance was provided by processing requests for taxonomic reidentifications and nominations of accessions to the inactive file. In total, 43 accessions received taxonomic re-identifications and 1,778 accessions were inactivated. The Maize CGC approved the inactivation of the Mangelsdorf-Galinat collection (1544 accessions). Inactivations included 92 accessions due to failure to germinate, and 241 accessions due to duplication; some of these accessions were also part of the Mangelsdorf-Galinat collection.

Additionally, 441 accessions were assigned PI numbers, 209 of which were *Zea mays* accessions from various collections including but not limited to: Goodman lines, GEM

releases, and CML lines. Crops curated by David Brenner were assigned 230 PI numbers, including 134 umbels, 52 pseudocereals, and 37 Melilotus accessions.

The NCRPIS continues to work on a project to digitize all paper documentation related to accession provenance, management, and performance. In total, 1,076 documents were uploaded to the GRIN database. This included 31 new documents filed in 2019 as well as 905 accession cards from the numerical card catalog and 104 inactivation forms. The card catalog contains valuable passport information and inventory growouts for accessions that were received prior to GRIN-Global implementation.

Content of the 'seed availability list's and miscellaneous login books and accession information forms were completely digitized in 2019. All new documentation, including passport files are being digitally maintained. We recorded important identifying information (Accession, Received Date, etc.) from the documents in Excel file format. The Excel files will enable us to rename files en-mass to conform to document naming conventions that more easily support future upload to the GRIN-Global database.

D. Order processing (S. Estrada)

The GRIN-Global public website has improved accessibility to germplasm information and the ability to search for desired crop characteristics. This year, the order processing team continued to refine the use of GRIN-Global order actions, attachments, and local order numbers in conjunction with Excel workbook templates to monitor order progress, streamline processing, and inform internal and external cooperators of order status. Order actions allowed both NCRPIS teams (curatorial personnel, seed storage, pathology) and other NPGS personnel (i.e., APHIS, GRIN-Global feedback) to more easily monitor a germplasm order as it progresses through the pipeline towards fulfillment. Documentation related to orders is attached directly to the corresponding GRIN order via the Order Wizard's attachment tab, thus accessible to internal NPGS users. External users may also add attachments (usually an import permit, shipping instructions, or Excel file request list) through their public website order history. These processing improvements are exceptionally useful for communication and management of additional documentation that is required for international germplasm distribution.

During 2019, 2531 orders for NCRPIS collection germplasm were entered into GRIN-Global. Of these, 2086 entered the order processing system via the GRIN-Global Public Website. We continue to see a high proportion (30-40%) of non-research, non-educational (NRR) orders being submitted for consideration. About 2,483 orders containing 86,076 items were processed in 2019. A detailed summary of NCRPIS distribution activity is summarized in the table below which illustrates various internal use purposes, and in Appendix Table 3. Over 57,500 items were shipped to external cooperators even though order processing was halted in January as a result of the partial government shutdown.

	2019 - NCRPIS Germplasm Distributions Summary										
		Grand Total						Distribute	d		
	Order Type	Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order	Orders	Orders (%)	Order Items	Items (%)	Avg. Items per Order
	Distribution	1,416	57%	70,185	75%	50	1,287	78%	56,857	83%	44
a	Non-research, non-educational	708	28%	11,858	13%	17	3	0%	3	0%	1
External	Observation/evaluation	37	1%	643	1%	17	36	2%	641	1%	18
ũ	Repatriation	6	0%	26	0%	4	6	0%	26	0%	4
	Total	2,167	87%	82,712	88%	38	1,332	81%	57,527	84%	43
	Backup	18	1%	765	1%	43	17	1%	635	1%	37
	Germination	189	8%	5,737	6%	30	185	11%	5,733	8%	31
nternal	Herbarium/reidentification	18	1%	156	0%	9	18	1%	156	0%	9
Inte	Phytosanitary Testing	51	2%	3,490	4%	68	49	3%	3,445	5%	70
	Replenishment/regrow	45	2%	929	1%	21	44	3%	918	1%	21
	Total	321	13%	11,077	12%	35	313	19%	10,887	16%	35
	Grand Total	2,488	100%	93,789	100%	38	1,645	100%	68,414	100%	42

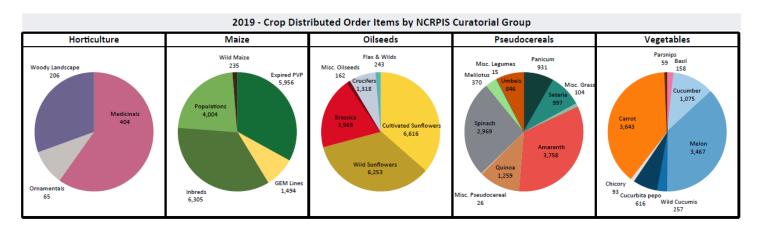
Shipped orders:

We processed 2,167 external orders. Of these, 1,332 (61%) were shipped and 835 orders cancelled. External orders were cancelled for a variety of reasons including: 705 (86%) were NRR, 71 requestors were unable to secure an import permit, and 39 for other reasons such as mistakes, duplication, NCRPIS was unable to satisfy phytosanitary restrictions, or lack of response activity from the requestor for a year or more.

Domestic orders accounted for 77% of all distributed orders and 56% of the distributed items (Appendix Table 3), indicating that U.S. requestors received fewer items per order. Maize was the most highly distributed crop within the United States. Multiple large orders for oilseeds dominated international distributions.

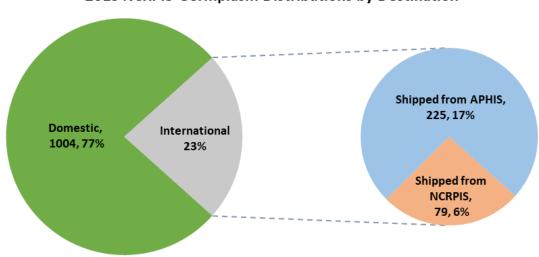
2019 - NCRPIS External Germplasm Distribution Summary by Curatorial Group							
	Orde	Order	ltems	Avg. Iten Orde			
Curatorial Team	United	International	United States	International	United States	International	
Horticulture	116	9	516	164	4	18	
Maize	575	147	12,169	5,941	21	40	
Oilseeds	175	84	6,228	11,933	36	142	
Pseudocereals	141	54	8,125	3,150	58	58	
Vegetables	99	57	52	74			
Grand Total	1,008	331	32,207	25,387	32	77	

Order distributions were also summarized by curatorial group, as seen in the pie charts below. Horticulture Oilseeds, and Vegetables saw high demand for a few crop maintenance groups while Maize and Pseudocereals requests were more balanced across crop groups.



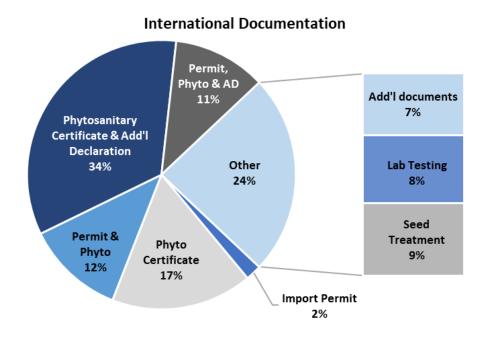
International orders accounted for 23% of those shipped and for 44% of all distributed items. Approximately one-fourth of all international orders were shipped directly from the NCRPIS. The remaining three-fourths of requests were transferred to APHIS in Beltsville for phytosanitary certificate issue prior to export. International orders shipped through APHIS/Beltsville increased in the final quarter of 2019 after the European Union issued new regulations requiring that all plant shipments be accompanied by a phytosanitary certificate.

For a more detailed view of orders, distributed external orders are visualized in the context of destination.



2019 NCRPIS Germplasm Distributions by Destination

Each country has unique restrictions regarding the importation of plant material. Therefore, a considerable amount of effort and documentation is required to process these international requests. We adapted the local order number field to distinguish shipping destination as well as serve as a 'quick reference' for documentation and additional quality assurance needed prior to dispatch of an order.



The order processing team was busy this year with many large requests for international germplasm distribution. Seventy five percent of all international orders required issue of a phytosanitary certificate prior to export and 25% of international distributions were shipped with an import permit. Import permit restrictions vary in complexity.

E. <u>Seed Storage (L. Burke)</u>

The seed storage area was staffed by one full-time, permanent federal employee (Lisa Burke), a three-quarter time temporary employee and five part-time student employees during 2019. In November, Ashley Sonner joined the seed storage as a fulltime, permanent USDA-ARS Biological Science Technician. Ashley had worked in seed storage and the germination lab as a part-time, temporary (limited appointment) employee during the previous two years.

We stored 1689 inventory lots, including 867 original seed lots. Of the increase lots, 672 were produced in Ames and 150 were produced outside of Ames. Across all stored inventory seed lots, we sampled and reviewed seed quantities of 2757 lots, and any discrepancies with GRIN information were corrected in the GRIN database. We prepared and transferred 1039 samples to the -20C freezer for long-term storage.

We filled 1337 seed orders in 2019, including those for distribution, observation, germination, transfer and backup. The NCRPIS distributed 47179 packets to meet distribution and observation requests. There were 632 lots sent to the National Laboratory for Genetic Resources Preservation (NLGRP) for backup, involving both

accessions new to the NLGRP and additional seed quantities for previously deposited accessions.

With the aid of our student workers, we prepacked 33914 packets from 3142 inventory lots. Prepacking increases efficiency of seed storage operations by speeding up order fulfillment and also helps keep the on-hand inventories more accurate. Prepacking also reduces the need to review total seed counts for individual accessions because distribution lots are continually monitored and only reviewed when order activity is high for a given accession.

In 2019, scanning of original samples was postponed until the vacant seed storage technician position was filled. Ashley Sonner has, since starting the seed tech position, participated in our imaging committee's meetings and will restart the imaging of original seed in 2020.

2019 was an active year for assigning PI numbers to accessions that were assigned Ames numbers; 1437 inventory samples were relabeled and moved to the chronologically correct location.

Lisa Burke continued to participate in the development of GRIN-Global. She served on the GRIN-Global Advisory Committee as chairperson. She chaired 19 meetings in 2019, each running between 1 and 2 hours. During the meetings, progress on curator tool and public web site enhancements were discussed, priorities were established, and the functionality of new software products verified. Continued focus was made on clarifying codes and adding descriptions for proper code usage.

Lisa assisted the RL and station's Program Manager in the past year with assessment of space needs and design for a new -20C cold storage building. The Midwest Area has made our new seed storage building a high priority, as ability to store at -20C will greatly extend the longevity of seed viability for the vast majority of our crops. This will reduce the frequency of seed regeneration, thus preserving seed, financial and personnel resources.

Lisa Burke continued as the station's CPR/AED/First Aid instructor. She provided two—year First Aid certification for 34 NCRPIS student workers and two-year CPR/AED/First Aid certification for 19 staff members. Each session was entered into the National Safety Council database and certificates of completion provided for each participant. Cooperative efforts with campus staff to improve the CPR/AED/First Aid training continues.

Lisa Burke participated in several outreach activities related to STEM and seed saving activities for 4H, home gardeners and Native American farmers. As leader for the Boone County Science and Tech club she guides club members in STEM activities. She also has developed a Power Point presentation called "Seed Saving for the Home Gardener" which highlights the process of controlled pollination, seed cleaning and preservation based on activities used at the station to preserve germplasm.

F. Germination (L. Pfiffner)

The germination lab was staffed by one full-time federal employee (Lisa Pfiffner) and up to three part-time student employees.

In 2019, the germination lab completed germination or Tetrazolium (TZ) testing on 196 orders containing 6,241 accessions.

Type of Order	Number of Orders	Number of Accessions
Regeneration	73	1,273
Maintenance	67	4,103
Original	29	637
Re-germ	15	105
TZ	9	91
Experiment	2	12
Observation	1	20
Total	196	6,241

With the maintenance testing backlog number increasing every year and the lack of germination data on an aging seed inventory, the germination lab decided to approach maintenance testing differently starting this year. Seed testing amounts were reduced to 50 or 20 (for maize) seeds per germination test, on crops approved by the curator. In the past, maintenance tests used 200 seeds per test. This change has allowed the germination crew to provide data on more accessions and a wider spectrum of species. After each reduced testing order, results were compared to past test results and if there was a discrepancy, the seed lot was tested again at 200 seeds. This year, 1,780 more accessions were tested than the previous year. Below are the crops and amounts that were tested at reduced seed amounts during 2019.

Crop	Number accessions tested with reduced seed amounts
Cuphea	67
Panicum	145
Brassica	709
Linum	994
Maize	682
Cultivated Sunflower	298
Total	2,895

Below is a table showing the number of accessions with recent germination tests, require testing, and average number of accessions tested annually per crop.

Crop	Number of Accessions	Number of accs with recent germination data 2010-2019	Percent of accs with recent germination data 2010-2019	Percent of accs requiring germination testing	Average Number of accs tested annually for germination 2010-2019
NC7-grass.echinochloa	315	273	87%	13%	26
NC7-grass.misc	142	80	56%	44%	10
NC7-grass.panicum	936	454	49%	52%	48
NC7-grass.setaria	1115	329	30%	57%	40
NC7-legume.melilotus	1006	183	18%	82%	11
NC7-legume.misc	299	50	17%	83%	9
NC7-pseudocereal.amaranth	3337	1870	56%	44%	151
NC7-pseudocereal.celosia	60	36	60%	40%	2
NC7-pseudocereal.perilla	25	24	96%	4%	3
NC7-pseudocereal.portulaca	13	10	77%	23%	1
NC7-pseudocereal.quinoa	451	261	58%	42%	36
NC7-spinach	413	304	74%	26%	27
NC7-umbels	1196	650	54%	46%	60
NC7-medicinals	1084	470	43%	57%	30
NC7-ornamentals	767	342	45%	55%	8
NC7-woody.landscape	2028	203	10%	90%	35
NC7-asters	450	72	10%	90%	6
NC7-brassica	2019	1693	84%	16%	196
NC7-crucifers	1307	557	43%	57%	20
NC7-cuphea	638	72	11%	89%	7
NC7-euphorbia	210	65	31%	69%	3
NC7-flax	2834	2818	99%	1%	244
NC7-flax.wilds	167	37	22%	78%	4
NC7-sun.cults	2322	420	18%	82%	201
NC7-sun.wilds.ann	1692	817	48%	52%	69
NC7-sun.wilds.per	899	359	40%	60%	21
NC7-maize.coix&tripsacum	53	8	15%	85%	4
NC7-maize.gems	354	322	91%	9%	4
NC7-maize.inb	2684	2146	80%	20%	29
NC7-maize.pop	16981	4267	25%	75%	52
NC7-maize.pvp	491	483	98%	2%	8
NC7-maize.teosinte	439	38	9%	91%	3
NC7-chicory	285	244	86%	14%	14
NC7-cucumis.cucs	1401	1332	95%	5%	109
NC7-cucumis.melo	3224	1047	32%	68%	36
NC7-cucumis.wilds	318	207	74%	26%	13
NC7-cucurbita	979	723	74%	26%	71
NC7-daucus	1563	1230	79%	21%	150
NC7-ocimum	106	99	93%	7%	12
NC7-parsnips	73	59	81%	19%	8

An experiment was done in coordination with Dr. Kevin Keener's group on the ISU campus. The objective was to determine the impact of atmospheric cold plasma treatment (ACP) on germination, vigor, and seedborne microbes. Crops tested were Chenopodium berlandieri var. zschackei, Daucus pusillus, Hypericum perforatum, Linum usitatissimum, Zea mays subsp. mays (two different accessions). Seed lots were selected for their dormancy, low germination and seed borne pathogen issues. Seeds were treated with the cold plasma at a certain strength for a certain amount of time. Both control and treated seeds were tested for vigor and germination. There were no significant differences between the control and the treated lots.

Participated in a call for data from Association of Official Seed Analysts (AOSA) in a National Survey-Data Collection Study to see the feasibility of using Tetrazolium Chloride (TZ) testing as an alternative viability test to the standard germination test. A *Zea* germination test had just been completed so TZ samples were pulled, tested and the results of both testing methods were sent to AOSA.

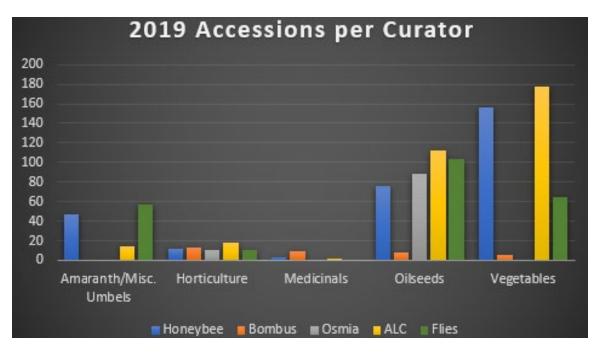
Germination protocols are based on AOSA protocols and from the Handbook of Seed Technology for Genebanks. If a taxon is not found in either of these references, then protocols are researched and devised for that genus/species. In this year, 31 protocols were created or modified.

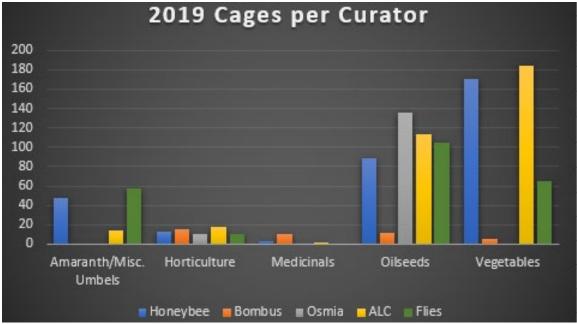
VII. CURATORIAL AND SCIENTIFIC TEAM REPORTS:

A. Controlled Insect Pollination Service Program (S. Hanlin, K. Judson)

Summary of Pollinators supplied to 2019 regeneration cages

Number of Unique ACCESSIONS per curator						
	Honeybee	Bombus	Osmia	ALC	Flies	TOTAL
Amaranth/Misc. Umbels	47	0	0	14	57	118
Horticulture	12	13	11	18	11	65
Medicinals	3	9	0	2	0	14
Oilseeds	76	8	88	112	104	388
Vegetables	156	5	0	178	65	404
OVERALL	294	35	99	324	237	989
OVERALL Number of TOTAL CAGE/I			99 Osmia	324 ALC	Flies	989 TOTAL
	HIVES per cui	rator				
Number of TOTAL CAGE/I	HIVES per cui Honeybee	rator Bombus	Osmia	ALC	Flies	TOTAL
Number of TOTAL CAGE/I	HIVES per cur Honeybee	rator Bombus	Osmia 0	ALC 14	Flies	TOTAL 119
Number of TOTAL CAGE/I Amaranth/Misc. Umbels Horticulture	HIVES per cur Honeybee 48 13	rator Bombus 0 15	Osmia 0 11	ALC 14 18	Flies 57	TOTAL 119 68
Number of TOTAL CAGE/I Amaranth/Misc. Umbels Horticulture Medicinals	HIVES per cur Honeybee 48 13	rator Bombus 0 15	Osmia 0 11 0	ALC 14 18 2	Flies 57 11 0	TOTAL 119 68 16





Progress:

Caged pollination:

Bee pollinators (minus the alfalfa leaf cutting bee) were supplied a single time to 514 cages for controlled pollination of 428 accessions. Alfalfa leaf cutting bee and fly-pollinated cages are tabulated and reported separately due to multiple distributions of those insects to the same cages over the pollination season.

Honey bee pollination:

Honeybees were used to pollinate 294 accessions in the field.



2019 Honeybee Pollinator Deliveries to Regeneration Cages

Crop Group	Total # of Accessions	# of Genera	# of Accessions/Genera			
Misc. Umbels	47	2	43 Coriandrum 4 Foeniculum			
Horticulture	12	3	6 Spiraea, 4 Cornus, 2 Caragana			
Medicinals	3	2	2 Echinacea, 1 Monarda			
Oilseeds	76	2	70 <i>Helianthus</i> , 6 Euphorbia			
Vegetables	156	5	88 Cucumis, 35 Daucus, 23 Cucurbita, 6 Pastinaca, 4 Ocimum,			
Total	294	14				

Overwintering success: 53% of the 30 wrapped three story parent colonies left outside survived, 73% of the 40 two- and three-story parent colonies survived in the indoor wintering facility, and 17% of the 78 double story nucleus colonies stored in the indoor wintering facility survived. It was observed that most of the double story nucleus hives (45%) were lost prior to putting them into the indoor facility and 12% of the parent colonies were lost prior.



All three-story parent colonies were left outside during the winter 2018/2019 at three locations and were wrapped with 30# roofing paper. All parent colonies stored inside were removed from the room starting on March 15th, and all outside colonies unwrapped on March 21st. The nucleus hives were removed from the over-wintering room on March 15th. In the winter of 2019/2020, we placed 21 two story parent colonies, 7 three story colonies and 60 double story nucleus hives in the overwintering room. We left outside and wrapped 10 three-story parent colonies at the NCRPIS in the fenced area designated for hives. Twenty-four three-story parent colonies were stored at two other locations (10, 14, 10 colonies per site). Five three-story colonies were lost prior to wrapping.

We purchased 60 five-frame nucleus hives from two local suppliers and 50 Italian queens from a California supplier to supplement over-wintering losses and to supply spring nucs used for cage pollinations. The queens arrived by USPS in early May, the nucs were picked up and put into full colony equipment in mid – May, allowing the honeybees to increase in population until late May before producing nucs from them. The hives were given three feedings of high fructose corn syrup (HFCS) and two pollen patty treatments during the buildup period. The caged queens were placed in nucleus boxes with two frames of brood and a single frame of honey and adhering bees.

In early May we selected queens from eight resilient, over-wintered parent colonies and set them up as "cell builder colonies" for queen production during the summer 2019. During the summer, however three of the hives were switched because they were too weak and wouldn't be able to be used for grafting. We made our first graft May 14th, knowing that with the first attempt we generally have only approximately 50% success. We could use the purchased queens to supply most of the nucs we were going to make at this time. Our average for the months' grafting was 19 queen cells per week, with nucleus hives produced until the end of May. In 2019 we had no issues with aggressive bees with our locally purchased bees. Of the purchased queens, only one was lost, even though some were held in queen banks for almost two weeks. All nucs which were not used in cages for pollination were fed HFCS, an additional super was placed below, and they were treated for mites to prepare them for over-wintering.



Image displays a single grafting frame with 55 pulled queen cells.



All hives were wrapped in tar paper early December to help protect and retain heat throughout the winter. These hives were fed syrup into early December, but most of the colonies and nucs went into winter lighter than in 2018 and will need to be fed sometime in February/March to assist with survival. The mortality rate prior to placing hives into the over-wintering room was 12% for the parent colonies and 45% for the nucleus hives.

Mite counts were made using the powdered sugar roll method in mid-June on 50% of the total colonies and double story nucs. Mite numbers were found to be between 0 to 10 mites per 100 bees, much lower than recent years. Most hives showed between 4 to 10 mites per sample, which is equal to the documented economic injury threshold level (EIL) of 5 mites per 100 bees. In August and September all colonies and nucleus hives were treated with Formic Acid (Mite Away Quick Strip®). Even though mite populations were lower, most hives went into winter with counts of 1 to 3 mites per 100 bees.

Starting in March through early April 2019, all parent colonies and nucleus hives were given five feedings of HFCS. In October to early November, all hives were fed six feedings, but because the medication used to treat dysentery nosema) Fumagilin – B® is not available at this time, all feedings were non-medicated. During the summer neither European Foul Brood (EFB) nor American Foul Brood (AFB) were observed. Three treatments of ApiLife-Var were used in the fall, an alternative product for Varroa treatment. Three treatments of Mite-away quick strips containing formic acid was used on the 10 colonies to kill and prevent the spread of Varroa mites.

As with the past three years, for wax moth control during the summer, all stored supers with "cleaned" frames were stacked at right angles to each other to prevent adult moth migration and to allow light in to reduce wax moth propagation within the equipment room. Starting in June through October, the lights in the equipment room were left on during working hours (8 hours; five days). All equipment removed from the field as "dead hives" was stored in the overwintering room at a temperature of 60° F. An additional air conditioner was installed inside the overwintering room for precaution.

Use of the new tank system proved to be more efficient, easier to maneuver, and we will continue to use the bulk tank in later years. We continued to use the 30-gallon poly "mixing" tank for filling feed containers. To prevent crystallization, insulated blankets were used to cover two tanks containing syrup and used a tank heater in the third. On May 20th, 852 gallons of HFCS were purchased for supplemental feeding during the summer and into the spring of 2020. We continued to use 5-gallon buckets in the spring and fall for refilling feed containers in the field in order to reduce container damage and syrup waste.

As in 2019, hive registration with the Iowa Department of Agriculture and Land Stewardship (IDALS) was done using "FieldWatchTM". FieldWatch allows you to register yards by plotting them directly onto Google maps. Unlike 2018, the locations only had to be confirmed in the system that they would continue to be used in 2019 in order to register them. The IDALS registry assists pesticide applicators in locating bee-yards and in obtaining contact information of appropriate beekeepers prior to spraying.

Bombus pollination:

Thirty-five "mini-research" colonies of *Bombus impatiens* were purchased from a commercial supplier and used to pollinate 43 field cages with 35 accessions. In 2019, we continued to use the queen-less "mini-research" hives for pollination. For some cages loose worker bees had to be retrieved and released when the hive was switched to a different location because the bees would not go back into the hive. While in storage, we would place sugar-soaked cotton wicks into holding containers for loose bees to feed on prior to release. A single *Bombus* hive can be used for pollinating more than one cage with a minimum lapse of 24 hours between sites to prevent pollen contamination.



In the *Cucurbita* cages in 2019, because of the amount of vegetation, a single *Bombus* hive was combined with a honeybee nuc for more complete pollination in each cage.

2019 Bombus Pollinator Deliveries to Regeneration Cages

	olo Bontono I ommator	Deliveries to	negeneration eages
Crop Group	Total # of Accessions	# of Genera	# of Accessions/Genera
Horticulture	13	3	6 Caragana, 6 Staphylea, 1 Cornus
Medicinals	9	1	9 Monarda
Oilseeds	8	1	$8\ Helianthus$
Vegetables	5	1	5 Cucurbita
Total	35	6	



We continued to use 60-quart protective plastic containers to house the cardboard *Bombus* hives while in field cages. Two water-filled quart containers are placed inside as weights to prevent the wind from blowing the container and hive off the stand. In some cages, the plastic container was replaced with two full honeybee supers taped together, and a telescoping lid placed over one end. For these shelters, no water bottles were needed because the weight of the boxes prevents them from blowing over in the wind. The protective shelter, bottles and hive are placed on a

full-size honeybee hive body and telescoping lid for a stand. The stand prevents the tub and hive from getting flooded in fields where standing water can occur.

Osmia cornifrons/O. lignaria pollination:

Osmia sp. were used to pollinate a total of 147 field cages and 1 greenhouse cage with 99 accessions.

2019 Osmia Bee Pollinator Deliveries to Regeneration Cages

Crop Group	# of Cages	Total # of Accessions	# of Genera	# of Accessions/ Genera
Horticulture	11	11	3	7 Staphylea, 2 Aronia, 2 Cornus
Oilseeds	136	88	2	87 Brassica, 1 Camelina
Total	147	99	5	



In the 2018 growing season, we obtained an increase of ca. 206 *Osmia* pupae (22 domiciles at 25 bees/domicile) which could be used for pollination and increase during the 2019 pollination season. We purchased from two suppliers as an outreach to new commercial suppliers. 1,000 Osmia cells were purchased from a new supplier from Idaho, and an additional 2,800 Osmia cells were purchased from the previous supplier in Washington in the spring of 2019. The Osmia supplied from Idaho arrived too

late creating some problems with emergence. This is an easy fix and did not discourage us from ordering from them in the future.

As in 2018, the pupae were shipped as loose cells prior to domicile placement in the field, 20 pupae (10 males and 10 females) were placed into specimen cups and were transferred into the domicile just before hanging it in the cage. For the greenhouse cages, six total pupae (10 male and 15 female) were placed into each cage. We also purchased an attractant to possibly increase our *Osmia* "increase" numbers at our orchard locations and to help keep the bees we were releasing "close to home". *Osmia* pupae were used to fill 177 two-inch domiciles. The two-inch domiciles were divided in the following manner, 147 were used in pollination cages and 30 were used at a single "increase" site.

In the fall of 2019, we collected ca. 605 pupae (25 domiciles at 25 bees) which will be used in the spring of 2020. This attractant appeared to work, as our numbers greatly increased compared to previous years. Additional pupae will be ordered in the spring of 2020 to assure enough pollinators for the spring cages and for placing at "increase" sites. 1,000 price/unit of Osmia were delivered on March 4th.

Alfalfa leafcutting bee (ALC) Megachile rotundata:

ALC bees were purchased as larvae in leaf cells from a single supplier for use in 2019, arriving in Ames, IA on February 6. The bee cells were held in refrigerated storage until scheduled for placement in warm incubation and bee emergence boxes. Bees were available weekly throughout the year for use in plant regeneration cages in the field and greenhouse from March 2019 through early November 2019. The 2019 pollinations were all completed using the 2019 cocoons for emergence of adults. The correct amount of Osmia was purchased in 2019 and lasted until February of 2020.



2019 Alfalfa Leafcutter Pollinator Deliveries to Regeneration Cages

Crop Group	# of Deliveries	# of Cages	# of Locations	# of Accessions	# of Genera	Time Period
Misc. Umbels	57	14	1	14	1	June – Aug.
Horticulture	80	18	4	18	5	April – Aug.
Medicinals	16	2	1	2	1	June – July
Oilseeds	846	113	5	112	7	Nov. (18) – Sept.
Vegetables	1583	184	5	178	4	Nov. (18) – Sept.
Total	2582	331	16	324	18	Nov. (18) - Sept.

In 2019, 2,582 total ALC deliveries were made to fourteen fields and three greenhouses with 331 cages containing 324 accessions. 655 cages received ALC for the year. A total of five greenhouse cages were still receiving pollination at the transition from 2019 into 2020.

Numbers of active ALC-supplied cages and frequency of bee delivery vary seasonally and by cage structure/location and individual accession characteristics. In normal pollination situations, ALC bees/cells are only provided to crops in the field during the summer months. However, at the station ALC are used outside of the normal time frame. From March through June 2019, greenhouse cages were supplied weekly with bees in the spring and summer. The 2019 field requests for ALC bees started in late April and the number of weekly active cage increased rapidly through mid- August and then declined with the last field cages supplied through early-November.

In 2019, we received Canadian sourced cells, which have fewer parasites and parasitoids than found in U.S. cells. Because the EGC was out of commission, ALC were relocated and used all summer long in the precision incubator, a low temperature refrigerated incubator. No decline in adult bee emergence from the precision incubator was observed. By fall, the EGC was fixed and in use again.



From September to early November, ALC were placed into cages of *Helianthus sp*, *Diervilla* sp, *Spirea* sp and several field vegetables cages. Under normal conditions ALC are not the major pollinator of some of these accessions, nor are they used this late in the season in field cages. Because we had no fall greenhouse pollinations but were able to continue to emerge a low number of bees,

we placed them into field cages rather than discard them.

Flies (Blue Bottle Flies and Houseflies):

Fly pupae of two species (Calliphoridae and *Musca domestica*) were purchased from two suppliers and incubated for weekly use from late December 2018 through early November 2019 for greenhouse and field pollinations.

From early June through August, 10 orders of 20,000 house fly pupae were purchased and from November 2018 through September 2019, 227 cups of blue bottle pupae were purchased for late season greenhouse pollinations that will continue into 2020. In 2019, 1640 fly deliveries were made to 10 fields and three greenhouses with 239 cages, containing 238 accessions representing 13 genera.

2019 Fly Pollinator Deliveries to Regeneration Cages

Crop Group	# of Deliveries	# of Cages	# of Locations	# of Accessions	# of Genera	Time Period
Misc. Umbels	215	57	1	57	2	June – Oct.
Horticulture	33	12	4	12	4	March – Sept.
Oilseeds	706	105	4	104	5	Feb Sept.
Vegetables	686	65	3	65	2	Nov. (18) – Sept.
Total	1640	239	12	238	13	Nov. (18) - Oct.

An average of four greenhouse cages and an average of 26 field cages received flies weekly from December 2018 through early November 2019. Three cages of *Daucus carota*, one cage of *Sinapis arvensis* subsp. *arvensis*, one cage of *Camelina rumelica* subsp. *transcaspica*, and one cage of *Camelina sativa* began receiving blue bottle fly pupae in mid-December 2019 and transitioned into the 2020 season.

Because blue bottle flies work better at cooler temperatures and more cage requests were for the cooler greenhouse, only blue bottle flies were distributed weekly during the winter, spring and fall. During the summer, both blue bottle flies and houseflies were distributed weekly to greenhouse and field cages for pollination. Adult flies are re-supplied weekly to cages in order to ensure continued pollinator presence. Most cages which have fly pollinators introduced, also have other pollinators present to assure flower pollination based on promotion of insect



competition. During the summer, if there were excess fly pupae available, they were introduced to some accessions which lack favorable flowers for fly pollinators (such as sunflower or melons) to supplement the other pollinators. This decision was made by the curators and the entomology staff to fully utilize the fly pupae.

Tests:

Feeding sugar in the winter:

In the winter of 2019, to improve hive survival and to reduce the amount of excess HFCS that needed to be cleaned from the floors after hive removal, we tested feeding a mixture of HFCS and granulated sugar to the bees. This mixture was 40% HFCS and 60% granulated sugar creating a denser paste that was easy to apply to the hives and nucs. By feeding the bees this mixture, there was no mess to clean up from the excess HFCS and no solid granulated sugar was wasted. The bees seemed to consume this paste very easily to where we could have provided a second round of feed. By late January, it was observed that the parent colonies and nucs were able to benefit from this feeding method. The parent colonies and nucleus hives appeared to be healthier, more active, and consuming this mixture.

Branding equipment:



To assist in identifying the station bee equipment and as a deterrent for equipment theft, all woodware was branded with an identifying stamp displaying that the hives are property of USDA, ARS and providing contact information. In the winter of 2018/2019, all newly assembled foundation frames were branded. We will continue to brand other woodware in the future as a precaution.

Safety:

Chemical Inventory:

In Mid-November 2019, C. Hopkins updated the Entomology chemical inventory and sent it to ISU and the USDA.

Defensive Driving:

Because of the amount of time that the bee crew is off site and the number of cumulative miles during the summer, annual driving training is needed to refresh good driving habits. Defensive driving courses were taken on AgLearn by full-time entomology personnel to fill this requirement. S. Hanlin completed "Defensive Driving Fundamentals" on February 18 and "Distracted Driving" on March 6. K. Judson completed "Defensive Driving" on April 17.

Epi-pens:

Epi-pens are available at four sites within the NCRPIS for use in prevention of anaphylactic shock caused by bee stings or other allergic reactions and are refreshed annually. In early March, K. Grooms (ISU Occupational Medicine nurse) was contacted for discussion and approval of the Epi-pen website and training materials on "the signs of anaphylactic shock and the correct use of Epi-pens". S. Hanlin

provided the link to the Epi-pen website and a "training completion document" to all permanent staff to be completed by the end of March. There was discussion as to whether the website and current training materials filled all requirements. A PowerPoint presentation will be shown in spring 2020 training instead of past materials. All PI staff training records were filed at ISU Occ. Med, and K. Judson exchanged expired Epi-pens to for new pens at the ISU pharmacy on April 18th.

Presentations and Outreach:

K. Judson and S. Hanlin worked with J. Carstens in the spring researching which species of lepidoptera could be most suitable for pollinating Monarda. We decided that



use of the Pipevine Swallowtail would be best to observe which insect (Pipevines or Bombus) more effectively pollinated Monarda better using both in caged

environments. S. Hanlin researched on how to obtain a permit, applied for one, and obtained 12



cocoons. K. Judson researched how to mimic their natural environment in order to increase emergence by using wood glue to prevent damage to the chrysalises. Eleven out of the 12 swallowtails emerged and survived most of the summer.

On August 14th, Iowa State University's fall forestry class visited for a field trip. S. Hanlin and K. Judson spoke to one group of students and introduced them to the variety of pollinators used at the station. The focus was on selection of pollinators for use with specific plant taxa. Part of the hour was spent with J. Carstens, where he discussed the various woody species grown at the station, and a Q and A session was provided at the end of the tour.

K. Judson was a mentor for a Coon Rapids high school student throughout the summer and fall. This student was taught the makeup of the hive, what each caste of honeybees does, how bees produce honey and wax, and the various equipment

Plans for 2020:

Feeding sugar in the winter:

In 2019, we tried feeding a mixture of HFCS and granulated sugar to nucs in the form of a patty or more solid liquid fee, this was fed to all honeybees. For the parent



colonies, we purchased a "winter patty" which consists of pollen, assorted nutrients and HFCS. The "winter patties" were consumed by the parent colonies that were stored inside and outside the OWR (over wintering room) but not the nucs. This was a cleaner method than feeding straight HFCS. The colonies and nucs were able to consume this mixture. For 2020, we will test feeding a mixture of HFCS and granulated sugar to nucs and colonies both inside and outside the OWR. This mixture will

come in a block form known as 'candy board' and is a typical feeding method used by most beekeepers. A box will be constructed in order to set the candy board onto the hive. The candy board will be neither a liquid nor solid. It will be hard but soft enough to consume and will not leave a mess. The idea is that the moisture and heat given off from the bees will help soften up the candy enabling the bees to consume it easier inside and outside during the winter months.

Additional:

We worked with K. Kabriel this summer making documentaries of the multiple pollinators we work with. Each documentary walked through the sourcing of pollinators, whether we raised or purchased them, pollinator and crop pairings, domicile type, whether pupal holders should be used based on the pollinator, and types of hives used at the station. During summer of 2020, we want to improve these documentaries. Each pollinator will be fully observed, queen rearing explained step by step, and splitting hives will be better demonstrated, improving the quality of the films. These documentaries will be used to show future workers what we do in full detail and possibly provided as part of online training.

Mite treatments for 2020 will change from use of ApiLife-Var to Oxalic Acid. This will be used as a drip technique in the fall when there is little to no brood present. We will compare our powdered sugar mite checks from the previous year to see which treatment does a better job in the removal of Varroa mites.

B. Plant Pathology (A. Testen, N. Pal)

Research:

Chlorine gas treatment of *Cucumis* seedlings:

Efforts continue to identify methods to reduce seedborne *Acidovorax citrulli*, the causal agent of bacterial fruit blotch, in *Cucumis melo* seeds. Melon seeds were exposed to chlorine gas in a vacuum desiccator for 12 hours. The seed treatment protocol was adapted from a protocol to disinfest soybean seeds. For each trial, 50 seeds were treated with chlorine gas and 50 seeds were not treated. Five trials were conducted to determine if chlorine gas would reduce incidence of bacterial fruit blotch symptoms in greenhouse grow-out bioassays. Chlorine gas did appear to reduce development of bacterial fruit blotch symptoms (Table 1) but getting consistent symptom development was difficult. Enzyme linked immunosorbent assays (ELISA) were used to confirm *A. citrulli* infection in two trials. In one trial, *A. citrulli* was only detected in control seedlings of one accession by ELISA but was not detected in treated seedlings. In another trial, *A. citrulli* was detected in control seedlings of two accessions but was also detected in one of three subsamples of treated seedlings from those accessions.

In one trial for accession PI 525148 18ncai02 SD, all control seedlings had damping off due to an oomycete but treated seedlings did not have damping off. This indicates that chlorine gas may be useful in reducing other diseases as well. Chlorine gas may reduce germination if seeds are not planted immediately following treatment, but this will be examined more systematically.

Table 1. Bacterial fruit blotch development following chlorine gas treatment in greenhouse bioassays

	# of accs	# of accs in which symptoms	# of accs in which symptoms
	in trial	did not develop in either	developed in control but not
		treated or control seedlings	treated seedlings
Trial 1	2	1	1
Trial 2	3	0	3
			(Symptom development was
			limited however due to overly
			hot conditions)
Trial 3	3	1	2
			(Acidovorax confirmed in only
			one accession by ELISA)
Trial 4	3	1	2
			(Acidovorax confirmed in two
			accessions by ELISA, one of three
			subsamples of treated seedlings
			from each accession also tested
			positive for <i>Acidovorax</i>)
Trial 5	3	3	0
		(Symptoms did not develop)	

Characterization of *Acidovorax citrulli* strains affecting the NPGS melon collection: There are two distinct genetic groups of *Acidovorax citrulli* that cause bacterial fruit blotch. These groups differ in their susceptibility to antibiotics, aggressiveness, and host preference. There are 56 *A. citrulli* isolates from NPGS accessions isolated by C. Block, and all were tested for pathogenicity and genetic group. We initially sought to characterize the *A. citrulli* isolates through BOX-PCR, but high variability within strains led to use of a group-specific PCR (Zivanovic and Walcott 2017). Fifty-five isolates were characterized as Group I and were pathogenic on both melon PI 601164 'Aurora' and watermelon 'Peacock.' One isolate was non-pathogenic and determined to not be *A. citrulli*. Two isolates from non-NPGS sources were confirmed to be Group II and will be used for comparison to Group I isolates in future trials.

Atmospheric cold plasma:

Atmospheric cold plasma is a plasma-technology that has been examined for reduction of foodborne diseases and may have applications as a seed treatment for breaking dormancy, improving germination, and reducing microbial load. In collaboration with Dr. Kevin Keener (Iowa State Department of Food Science and Human Nutrition, now University of Guelph), we conducted a pilot study to look at the impact of atmospheric cold plasma on seed germination and seed associated microbes of seeds within the NCRPIS collection. Six accessions were selected for testing, representing five genera, Daucus pusillus, Chenopodium berlandieri var. zshackei, Zea mays, Linum usitatissimum, and Hypericum perforatum. These accessions were selected based on issues with dormancy (Daucus and Hypericum), poor germination (Zea and Linum), and seedborne pathogens (Hypericum).

Seeds were exposed to an indirect atmospheric cold plasma treatment for 60 seconds and were kept in the treatment bags for 24 hours to maintain exposure to any reactive molecules. Following treatment, subsamples of seeds were tested for germination

while other subsamples were used for determination of bacterial and fungal colonization. The subsample of seeds used for microbial colonization was later used to measure impacts on seedling vigor. We did not observe a consistent significant impact of cold plasma treatment on germination, vigor or microbial populations. This was a pilot study, and while we did not see significant impacts, there are many variables, such as voltage, treatment time, carrier gas, that can be investigated to improve atmospheric cold plasma as a seed treatment.

Fungicide seed treatments for cucurbits:

Damping off of *Cucumis* and *Cucurbita* seedlings has been an ongoing issue in Cucurbit increases at the NCRPIS. A study was conducted to assess the impacts of three fungicide seed treatments on *Cucumis* seed germination. The fungicide treatments used were Apron XL plus Maxim 4FS, Dynasty, and Thiram. These treatments were compared to a non-treated control. Four accessions were compared representing both newer seedlots (2017 increases) and older seedlots (1996 increases). Use of fungicides did not decrease seed germination rates and should be used in future Cucurbit increases.

<u>Unknown Pseudomonas from Turkmenistan melon collection:</u>

In 2009, Charlie Block isolated a set of bacteria causing foliar diseases on melon obtained from a 2008 collecting trip to Turkmenistan. These unknown bacteria were not identified to species and may be novel pathogens of melon. We tested 12 isolates of these bacteria biochemically and by BOX-PCR to determine if they were all identical. Ten of 12 strains caused hypersensitive reaction on tobacco, and most of these strains were fluorescent on King's B medium. The ten isolates were then tested for pathogenicity on melon (PI 601164). Symptoms developed on all treated plants and bacterial streaming was observed indicating that all strains were pathogenic. These isolates will be identified by multilocus sequence typing in collaboration with Charlie Block.

Quinoa downy mildew qPCR:

Quinoa downy mildew, caused by *Peronospora variabilis*, is the most important disease of quinoa globally and is seedborne, contributing to its local and international spread. Diagnostic methods would improve our understanding of seedborne transmission and disease development. A standard PCR assay to detect *P. variabilis* in seed and plant tissue has been developed (Testen et al. 2014), but a quantitative PCR (qPCR) assay would improve sensitivity and allow for quantification of pathogen loads. Three SYBR green qPCR primer sets were developed based on the cytochrome oxidase II region (COXII). These sets amplified *P. variabilis*, but unfortunately also amplified DNA from the closely related spinach downy mildew pathogen, *Peronospora effusa*. Moving forward, primers and probes will be developed using the Taqman chemistry, which should improve specificity. Future work in this area will be conducted in collaboration with researchers at the University of New Hampshire, Colorado State University, and University of Arkansas.

New diseases in oilseeds and guinoa at NCRPIS:

During field inspections in 2019, two new diseases were observed in Ames, a phytoplasmal disease affecting flax and Euphorbia and anthracnose of quinoa. The

phytoplasmal disease was characterized as a 16SrI-B phytoplasma through 16S sequencing. The pathogens causing stem lesions on quinoa were identified as *Colletotrichum coccodes* and *Colletotrichum truncatum* through ITS sequencing. These represent first reports of these diseases and will be submitted for inclusion in *Plant Disease*.

Screening of GEM doubled haploids for Fusarium verticillioides resistance:

In collaboration with Jim Holland and Matt Krakowsky, the pathology team assisted with *Fusarium verticillioides* inoculation and Fusarium ear rot rating of 46 GEM doubled haploid lines. Disease development was poor in Ames (mean ear rot 16%), but good disease development occurred at the North Carolina sister site. Significant segregation for disease resistance was not observed at the Ames site, but significant segregation was observed at the North Carolina site.

Soil health:

The pathology team is working with Fred Engstrom and Jeff Schwartz to develop sampling protocols to improve soil health monitoring at the NCRPIS.

Phytosanitary Inspections of Seed Increases:

Phytosanitary inspections were conducted on 914 NPGS seed increases in 2019 (734 field increases and 180 greenhouse increases). In addition, phytosanitary inspections were conducted in 4,099 GEM plots. The pathology team worked with curation teams to ensure that disease issues were diagnosed and management recommendations provided in a timely manner. The number of accessions and total number of disease observations for phytosanitary inspections can be found in Table 2.

Table 2. Number of accessions and number of observations loaded to GRIN for 2019 for either field inspections or laboratory testing

	Phytosai	nitary inspections	Lab testing		
Стор	# of Accs	Accs # of Obs uploaded to GRIN		# of Obs	
Grasses	52	52	0	0	
Chenopods	36	36	0	0	
Amaranth	92	92	0	0	
Umbels, Legumes, & Misc.	53	53	0	0	
Brassica	86	86	0	0	
Euphorbia	6	6	0	0	
Flax	45	45	0	0	
Sunflower	122	732	1,187	4,783	
Maize	245	2,695	1,777	2,816	
Cucumis	93	651	98	98	
Cucurbita	24	161	25	25	
Daucus	50	50	0	0	
Ocimum	5	5	0	0	
Parsnips	7	7	0	0	
Total	916	4,671	3,087	7,722	

Maize and GEM:

Phytosanitary inspections were performed for 245 plots of maize and for 4,099 GEM plots. In general, disease severity was low in plots. Despite special scouting, tar spot was not observed at the NCRPIS. Crazy top was observed in four maize accessions. No other diseases of key phytosanitary concern were observed (Stewart's Wilt, Goss's Wilt, viral diseases, and head smut).

Oilseeds:

Phytosanitary inspections were conducted for 122 sunflower plots. Apical chlorosis and downy mildew were not observed in early season scouting. Sunflower downy mildew was observed in four plots in mid-season scouting, and the pathology team removed symptomatic leaves from each plot until symptoms were no longer observed. *Sclerotinia sclerotiorum* was found in one plot.

Flax and Euphorbia increase plots (51), in general, had very little disease. Phytoplasma symptoms were observed in 10 plots (nine flax and one Euphorbia).

For Brassica increases, black rot was observed in 18 of 86 plots.

<u>Vegetables</u>:

Squash mosaic virus (SqMV) screening was conducted on 124 accessions prior to planting. Three of 25 *Cucurbita pepo* accessions tested positive for SqMV, but no *Cucumis* accessions were positive.

For *Cucumis* and *Cucurbita* increases, disease severity was, in general, low. A spray program was instituted rotating between a powdery mildew-specific fungicide (Procure or Quintec) and a broad spectrum (Bravo or Quadris) fungicide in order to manage powdery mildew and anthracnose.

No diseases of phytosanitary concern were observed in carrot, basil, or parsnip increases.

Amaranthus, Chenopodium, Panicum, Setaria and Miscellaneous Apiaceae and Poaceae:

For phytosanitary inspections for the Amaranth curatorial crew, 95 phytosanitary inspections were conducted for winter greenhouse increases, 56 plot inspections were conducted for field increases, and 100 phytosanitary inspections for autumn greenhouse increases. For greenhouse increases, no diseases of phytosanitary concern were observed, with the exception of one *Setaria* accession infected by *Pyricularia* sp. (blast). For field increases, no diseases of phytosanitary concern were observed.

Seed Health Testing:

In 2019, 2,816 ELISA tests were conducted on 1,777 seed lots, while bioassays, plating, or microscopic observations were conducted on 1,187 lots (all *Helianthus annuus*). A summary of seed health testing by accession and number of observations can be found in Table 2. This seed health testing supported 51 orders along with internal testing for the maize curation and GEM teams. Maize seed health testing was the main focus of testing with many international orders requiring testing for Stewarts wilt, Goss's Wilt, Maize chlorotic mottle virus (MCMV), and Wheat streak mosaic virus (WSMV).

Additional declarations were written in support of phytosanitary certificates for 138 orders.

References:

Testen, A.L., Jiménez-Gasco, M.M., Ochoa, J.B., Backman, P.A. 2014. Molecular detection of *Peronospora variabilis* in quinoa seed and phylogeny of the quinoa downy mildew pathogen in South America and the United States. Phytopathology. 104:379-386.

Zivanovic M and Walcott RR. 2017. Further characterization of genetically distinct groups of *Acidovorax citrulli* strains. Phytopathology 107: 29-35.

C. <u>Amaranthus, Celosia, Chenopodium, Coronilla, Dalea, Echinochloa, Galega, Marina, Melilotus, Panicum, Perilla, Setaria, Spinacia and miscellaneous Apiaceae and Poaceae (D. Brenner, S. Flomo)</u>

Acquisitions:

We acquired 42 accessions: three Amaranthus, 11 Chenopodium, two Echinochloa, one Ligusticopsis, one Oenanthe, one Oxybasis, one Portulaca, 20 Setaria, and 2 Taenidia.

A diverse group of accessions were acquired in 2019. Dr. Jack Dekker donated germplasm in 2016 that was logged in to the collection in 2019. These 34 Dekker accessions (Ames 34403–34421, and Ames 35198–35212) were collected by Dekker to provide triazine herbicide tolerant and herbicide susceptible examples for weed physiology research. They consist of 3 *Amaranthus*, 10 *Chenopodium*, two *Echinochloa*, and 19 *Setaria*.

The Ligusticopsis wallichiana (Ames 35038) accession, a carrot relative, was part of a seed transfer from the Millennium Seed Bank. The Oeanthe (Ames 35213, an undescribed species collected in Iowa, was provided by Mr. Jimmie Dean. The Oxybasis urbica (Ames 34399) was a segregate from a Tampa, Florida Chenopodium accession of seeds of mixed species. David Brenner collected two accessions during travel to San Antonio, Texas for an agronomy conference: a wild Chenopodium of unknown species with dark red foliage (Ames 35258), and a Portulaca pilosa (Ames 35259) at the northern extreme its species range in Kansas. David also collected a Setaria viridis var. major (Ames 35056) with characteristically yellow-brown bristles to complete a set of all four bristle colors known in Setaria viridis of the Midwest. The two Taenidia integerima (Ames 34950 and Ames 34955) accessions are of a native species with aromatic foliage and yellow flowers and have ornamental potential; Jeff Carstens was helpful in acquiring these Taenidia.



Ames 35056 Setaria viridis var. major has distinctive yellow-brown bristles. The plants are anatomically between Setaria faberi which has larger seeds, and Setaria viridis var. viridis which has smaller heads on a smaller plant with more stems. The population was collected along weedy farm roads near Ames, Iowa.

In addition to the 34 accessions described above, 197 additional *Chenopodium* accessions were donated but are not yet logged in, so do not have accession numbers. These accessions were collected in the United States by Eric Jellen of Brigham Young University with Plant Exploration funding from the USDA/ARS, Plant Exploration Office, Beltsville, MD.

Collection Maintenance:

Second wave of regenerations:

Many of our seed increases are to replace older seed lots that have aged in storage. This is a result of active seed increasing of new accessions in the 1980s, and 1990s, requiring a second wave of regenerations 30 years later. If these accessions have temporary "Ames" numbers we are assigning new permanent "PI" identification numbers to replace the temporary numbers, so accessions are not regenerated twice with temporary numbers.

Chenopodium:

We are growing more wild species of *Chenopodium* now that the entire genus is a research priority for improving *Chenopodium quinoa*. Thirty-seven seed lots of wild Chenopodium were harvested in 2019. Our success propagating wild *Chenopodium* species has improved with a new dormancy breaking protocol involving breaking the seed coats with hand tools. Hailey Bryson, a student employee led the development of our dormancy releasing protocol.



PI 691469 *Chenopodium berlandieri*, abnormal seedling with a root growing from the side of the hypocotyl after the main tap root broke off. An empty seed coat is still attached to the cotyledons. These abnormal seedlings without tap roots are common with our new dormancy breaking protocol. They can establish healthy plants and produce normal seed harvests in greenhouse conditions.

Grasses:

Dr. Anna Testen, found blast, a fungal disease caused by *Pyricularia* species in a planting of *Setaria viridis* (Ames 34411) in October 2019. This is a seedborne disease. The diseased planting was destroyed, and a second planting in the winter of 2020, after a seed treatment, was disease free.

Miscellaneous Apiaceae:

The field cages plantings included 51 *Coriandrum*, and 4 *Foeniculum*. Also, 3 *Angelica* accessions were over-wintered in the field for anticipated 2020 harvests. An *Orlaya*, and a *Tordylium*, both obscure wild genera, were planted in the fall of 2019 for spring harvests in the greenhouse, they are both Mediterranean accessions adapted for winter-growing and spring-flowering.

Spinacia:

In 2019, 14 accessions were regenerated by Beiquan Mou in Salinas, CA, and one accessions was regenerated at the NCRPIS, a wild spinach (PI 647859). Fifteen accessions were sent to Beiquan Mou in Salinas, CA for regeneration in the 2019-2020 planting. The planting stocks were sent directly to Dr. Mou from the backup collection at the NLGRP in Ft. Collins, CO. We anticipate receiving the harvested seeds in mid-2020.

Characterization/evaluation/taxonomy:

The GRIN database was enhanced with updates listed below in the "Updating GRIN 2019" table.

Updating GRIN 2019

Count	Name	Description
22	Taxonomic Re-IDs	Taxonomic changes were made for accessions of six genera. Ten of the changes were in <i>Chenopodium</i> .
204	PI numbers	Permanent "PI" number identifiers were assigned to accessions with temporary Ames numbers. They include 98 <i>Coriandrum</i> accessions and a mix of other genera.
320	Citations	Links to research publications were loaded onto cited accessions in GRIN. The citations included: Waselkov et al. 2018. A phylogeny of the genus <i>Amaranthus</i> (Amaranthaceae) based on several low-copy nuclear loci and chloroplast regions, linked to 53 accessions.
4,514	Observations	Observations were loaded into GRIN for traits such as flower color, male sterility, and seed weight.
1,121	Images loaded	These include images taken by plant collectors during field work.
370	Seed lots stored	Backlogs of harvested seeds dating back to 2014 were made available for distribution in 2019.
22	Taxonomic Re-IDs	Taxonomic changes were made for accessions of six genera. Ten of the changes were in <i>Chenopodium</i> .

Amaranthus:

It is difficult for plant breeders to cross amaranth plants because the flowers are too small to manipulate. But we are developing a better way to make these crosses using cytoplasmic male sterility. In other crops such as sorghum and sunflowers, cytoplasmic male sterile systems allow the commercial production of F₁ hybrid seeds and boost agricultural production. The cytoplasmic male sterile amaranth line PI 686465 (DB 199313), developed by David Brenner, was released via GRIN and published in the Journal of Plant Registrations (Brenner, 2019). Dr. Markus Stetter at the University of Cologne will study the genetic basis of this male sterility with samples we sent there.

In 2019, PI 686465 was used for additional research as a female in crosses with ten male fertile accessions to evaluate the male's ability to maintain or restore male fertility. The outcomes of these crosses are publicly available in the Descriptors part of GRIN, listed in the MALESTERILE trait, and a summary was sent to an amaranth

email list so that many amaranth research projects are aware of this progress. Of the 10 males evaluated, eight maintained male sterility, and two restored male fertility. So, there are choices for expanding the use of male sterility with diverse amaranth backgrounds that can be evaluated for useful hybrid vigor.

Amaranthus Male Sterility Maintained or Restored

Male fertility outcomes in the F ₁ generation from crossing PI 686465 male sterile						
amaranth as the female parent and selected accessions as males						
https://npgsweb.ars-grin.gov/gringle	https://npgsweb.ars-grin.gov/gringlobal/methodaccession.aspx?id1=159032&id2=496276					
Maintained male sterility	PI 538255, PI 572260, PI 572263, PI 599338,					
Maintained male steriffty	PI 605354, PI 608791, PI 642741, PI 689685					
Restored male fertility	PI 576485, PI 689689					

<u>Chenopodium</u>:

In addition to imaging growing plants we are imaging leaves since they are often key traits in taxonomic descriptions. These images will be a useful online reference in GRIN to supplement to published prose descriptions in floras.



Ames 33012 Chenopodium neomexicanum leaf image.

Outreach and Presentations:

North American Wild Relatives of Grain Crops:

This book chapter (Brenner, Bockelman, and Williams, 2019) reviews the crop wild relatives of *Amaranthus*, *Chenopodium*, *Setaria*, and other genera. It provides an analysis of the diversity of these North American crop wild relatives, their distribution, status of germplasm collections, development needs, and targeted collection gaps. This chapter is part of a two-volume set on North American crop wild relatives written by knowledgeable NPGS and other public sector scientists.

Male sterile amaranth:

The first cytoplasmic male sterile amaranth accession (PI 686465, DB 199313) was developed at the NCRPIS. It is available for distribution and the release was published in The Journal of Plant Registrations (Brenner, 2019).

Release of DB 2008910:

We released a dwarf plant breeding line of *Amaranthus cruentus* (DB 2008910) via Iowa State University. A descriptive write up was posted online (Brenner, 2019b).

This amaranth line is a brachytic dwarf that grows about 65 cm tall and has internodes about 1.5 cm long. It is intended for crossing with standard amaranth lines to generate progenies that segregate for useful dwarf types. It may also be useful for leafy vegetable production since it has very little stem in proportion to the leaves.

Radio interviews:

David Brenner was interviewed about germplasm, for radio broadcasts, twice in 2019 (Brenner, 2019d, 2019e).

Crop Germplasm Committee reports:

Written progress reports were prepared for the Clover-Grass, New Crops, and Leafy Vegetable, Crop Germplasm Committees.

Service:

As Past Crop Science Society C8 Division (Genetic Resources) Chair in 2019, David organized a committee to select the outstanding genetic resources paper published in Crop Science in 2018 and chaired another committee for selecting of the speaker for the 2019 Calvin Sperling lecture: Dr. Pamela Soltis from the University of Florida.

Plans for 2020:

We have a new collaboration with Dr. Burton Johnson, of North Dakota State University. Dr. Johnson will grow and observe our amaranth plant breeding lines such a DB 2008910 (above) in his research fields near Fargo.

Loading old images into GRIN will be a priority in 2020. We have backlogs of unloaded accession images dating back to 2014 and hope to be current with image loading by the end of 2020.

Our field cages are prone to weed infestations. Typically, we weed these cage footprints carefully, and then additional weeds germinate from the seed bank in the soil. In 2020 we will experiment with two pre-emergence herbicides to control seeds that germinate after the first weeding. Both herbicides are granular, marketed as "Preen" and manufactured by Lebanon Corporation. One is an organic herbicide made from corn gluten meal "Preen Natural Vegetable Garden Weed Preventer" ®, and the other "Preen Extended Control" ® is a formulation of trifluran.

Some difficult to regenerate *Foeniculum* accessions are too long season for seed production after direct seeding in the field in our climate. They flower in late September and don't mature seeds before frost. In January 2020 we planted four of these accessions in a greenhouse and will determine whether these older plants will flower early enough for seed production after transplanting into the field.

Publications about our germplasm:

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http://aaasjournal.org/submission/index.php/aaas/article/view/66/35/66-Article%20Text-339-3-10-20180414.pdf

Brenner, D.M. 2019a. Registration of DB 199313, cytoplasmic male sterile grain amaranth genetic stock. Journal of Plant Registrations. 13:251–253. doi:10.3198/jpr2018.06.0042crgs

Brenner, D.M. 2019b. ISURF#04957: New dwarf leafy amaranth variety DB-2008910. https://www.cad.iastate.edu/files/page/files/new_amaranth_variety_release_0.pdf

Brenner, D.M. 2019c. Male sterile grain amaranth genetic stock. Crop Science Society of America News. 64:13. doi:10.2134/csa2019.64.S008

Brenner, D.M. 2019d. Crop wild relatives. Radio interview on KHOI radio by M. Meetz and M. Harris, 1600 am. Science Bytes. #40. November 2019.

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Brenner, D.M., H.E. Bockelman, and K.A. Williams. 2019. North American Wild Relatives of Grain Crops. In: S.L. Greene, K.A. Williams, C.K. Khoury, M.B. Kantar, and L.F. Marek (Eds.). North American Crop Wild Relatives. Volume 2: Important Species, (pp. 41–82). Cham: Springer. doi:10.1007/978-3-319-97121-6_2

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Hinojosa, L., N. Kumar, K.S. Gill, and K.M. Murphy. 2019. Spectral reflectance indices and physiological parameters in quinoa under contrasting irrigation regimes. Crop Science. 59:1927–1944. doi: 10.2135/cropsci2018.11.0711

Hinojosa, L., J.B. Matanguihan, K.M. Murphy. 2019. Effect of high temperature on pollen morphology, plant growth and seed yield in quinoa (Chenopodium quinoa Willd.). Journal of Agronomy and Crop Science. 205:33–45. doi: 10.1111/jac.12302

Hong, S., K. Cheon, K. Yoo, H. Lee, M. Mekapogu, and K. Cho. 2019. Comparative analysis of the complete chloroplast genome sequences of three Amaranthus species. Plant Genetic Resources: Characterization and Utilization. 17:245–254. doi:10.1017/S1479262118000485

Kuo S-m, Chen Y-r, Yin S-y, Ba Q-x, Tsai Y-c, Kuo WHJ, et al. 2018. Waxy allele diversification in foxtail millet (*Setaria italica*) landraces of Taiwan. PLoS ONE. 13(12): e0210025. doi.org/10.1371/journal.pone.0210025

Kysel, M., V. Stefunova, M. Bezo, J. Ziarovska. 2018. The effect of microsatellite core sequences in the differentiation of *Amaranthus cruentus* genotypes. Journal of Microbiology Biotechnology and Food Sciences. 7:607–610. doi: 10.15414/jmbfs.2018.7.6.607-610

Mangelson, H., D.E. Jarvis, P. Mollinedo, O.M. Rollano-Penaloza, V.D. Palma-Encinas, L.R. Gomez-Pando, E.N. Jellen, and P.J. Maughan. 2019. The genome of *Chenopodium pallidicaule*: An emerging Andean super grain. Applications in Plant Sciences. 7: e11300. doi:10.1002/aps3.11300

Maughan, P.J., L. Chaney, D.J. Lightfoot, B.J. Cox, M. Tester, E.N. Jellen, and D.E. Jarvis. 2019. Mitochondrial and chloroplast genomes provide insights into the evolutionary origins of quinoa (Chenopodium quinoa Willd.). Scientific Reports. 9:185. doi:10.1038/s41598-018-36693-6

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D. <u>Horticulture (J. Carstens)</u>

The Horticulture project currently holds 3,871 accessions representing 186 genera (Table 1.0). Significant NC7-medicinal collections includes: Actaea (44), Agastache (84), Echinacea (193), Calendula (83), Hypericum (226), Monarda (153), Prunella (54), and Tanacetum (54). Significant NC7-ornamentals collections includes: Alcea (34), Malva (55), Phacelia (53), Potentilla (127), Sphaeralcea (90), and Thalictrum (52). Significant NC7-woody.landscape collections includes: Aronia (104), Betula (170), Cornus (205), Euonymus (60), Fraxinus (495), Gymnocladus (89), Rhus (100), Salix (56), Spiraea, (103), Staphylea (45), and Ulmus (44). Jeff Carstens is serving as curator. The horticulture technician position remained vacant.

Table 1. Active accessions maintained in the NC7 horticulture collections (medicinals, ornamentals, and woody landscape) as of December 31, 2019

Management group	Genera	Accessions
NC7-medicinals	35	1084
NC7-ornamentals	54	767
NC7-woody landscape	97	2020
Total	186	3871

Acquisitions:

During 2019, we acquired a total of 124 accessions including 31 medicinal, 29 herbaceous ornamental, and 64 woody landscape accessions to the horticulture

collections. Unfortunately, 11 of these accessions (*Salix*) that originated from the Republic of Georgia were dead upon arrival. *Salix* seeds are extremely short-lived, especially at room temperature. Recollections on these accessions will be attempted in the future with a focus on expediting the seed processing and shipping process. Accessions of *Sassafras* (1) and *Fragaria* (1) were collected through the horticulture project and transferred to other NPGS genebanks.

Targeted acquisitions were focused on collecting *Monarda brevis* (5) in West Virginia completed by Jeff Carstens, Cole Hopkins (Iowa State University) and Jared Trent (NCRPIS); *Cladrastis kentukea* (6) in Missouri and Oklahoma completed by Carstens and Trent; and *Salix* (3) in Iowa completed by Carstens, Andy Schmitz (The Brenton Arboretum), and Trent.

A transfer of Seeds of Success accessions (62) largely included *Potentilla* (21), *Rhus* (20), and *Spiraea* (7), with additional taxa including *Agastache*, *Cornus*, *Dasiphora*, *Drymocallis*, *Monarda*, *Physocarpus*, *Scuttelaria*, and *Viburnum*.

Significant contributions from other cooperators included *Monarda* austroappalachiana (1) from Mark Pistrang (US Forest Service) and also *Monarda* serotina (2) sampled in Indiana and Missouri by Scott Namestnik (Indiana Department of Natural Resources) and Alan Brant (self-employed), respectively. Both *M. austroappalachaiana* and *M. serotina* represent germplasm holdings that are new taxa to the NPGS.

Collection Maintenance:

Regenerations:

Existing plantings that mostly consisted of *Monarda*, *Spiraea* and *Staphylea* were harvested via controlled pollinations. A total of 28 accessions were harvested.

We attempted germination for future regeneration on 24 accessions focused on *Aronia*, *Monarda*, *Rhus*, and *Salix* accessions.

A total of 24 accessions were transplanted to the field mostly focused on *Aronia*, *Monarda*, *Rhus*, and *Salix* accessions.

The first accession to be regenerated from utilizing an offsite location was harvested in 2019. This accession (Ames 33233) was planted in spring of 2017 (30 plants) resulting in a harvest of approximately 50,000 seeds.

Availability and Backup:

Currently, approximately 71% of the medicinals, 69% of the herbaceous ornamentals, and 52% of the woody landscape accessions are available.

Currently, approximately 76% of the medicinals, 78% of the ornamentals, and 47% of the woody landscape accessions are backed up at the National Laboratory for Genetic Resources Preservation (NLGRP) in Ft. Collins, Colorado.

The horticulture crew assumed the management of pollination screen repair on November 2018. The team utilizes a commercial sewing machine to repair torn seams and broken zippers. A total of circa 75 screens were repaired in 2019.

<u>Viability Testing</u>:

A total of 64 seed viability assessments were made for the horticulture collections including increase (19) and original (45). Viability data for 6 accessions were added to GRIN by duplicating viability records executed by NLGRP. This practice is being executed in order to reduce duplication of testing of accessions that were recently tested. In 2019, communication between NC7 and NLGRP was initiated in order to employ the use of identical testing protocols. Tests for *Spiraea* that previously used different protocol between NC7 and NLGRP are now following the same protocol.

Distributions:

Distribution figures for the horticulture collections are summarized in Table 2 and 3, below, and Appendix Table 3. For the combined horticulture program, we distributed 123 external orders to 99 requestors totaling 673 items from 502 accessions. We cancelled 233 orders from 182 requestors representing 1,417 items. Most of the orders were cancelled because they were requested for home gardening or other non-research use and/or commercial sources could meet the needs of the request. In addition to the 123 distribution orders, 18 orders to 6 requestors totaling 187 items from 53 accessions were for herbarium specimens sampled at NCRPIS or in nature. A significant number of *Monarda* were distributed for varietal development and genetic studies totaling 8 orders and 88 samples. *Hypericum* was largely distributed for anthropological studies (botanical reference collections) and *Agastache* for genetic marker development. A number of leaf tissue samples of *Cladrastis kentukea* were sent to Kevin Conrad at the Woody Landscape Plant Germplasm Repository for genetic analysis.

Table 2. Taxa most distributed from the NC7 horticulture program in 2019

Taxa	Most distributed (greatest to least)
Medicinals	Monarda
	Hypericum
	Agastache
	Origanum
	Echinacea
Ornamentals	Potentilla
	Malva
	Alcea
	Glebionis
	Lavatera
Woody landscape	Fraxinus
	Salix
	Cladrastis
	Rhus
	Aronia/Caragana/Ulmus

Table 3. External domestic and foreign germplasm distributions for the NCRPIS horticulture program 2015 through 2019

Crop	Year	No. of Orders	No. of Recipients	No. of Items Distributed	No. of Accs Distributed
Medicinals	2015	39	39	218	174
	2016	36	33	99	99
	2017	53	44	387	233
	2018	44	36	218	169
	2019	53	47	404	293
	Average	45	40	265	194
Ornamentals	2015	21	20	78	74
	2016	33	30	72	61
	2017	27	26	174	160
	2018	46	40	117	93
	2019	22	21	63	55
	Average	30	27	101	89
Woody Landscape	2015	95	66	335	191
	2016	97	69	302	168
	2017	71	56	367	146
	2018	82	68	327	164
	2019	72	58	206	154
	Average	83	63	307	165

Characterization/taxonomy:

During 2019, three horticulture accessions Salix (2) and Monarda (1), were renamed based on morphological characteristics. No PI numbers were assigned. Communication with taxonomist Melanie Schori (USDA-ARS), resulted in the elevation of Monarda fistulosa var. brevis to the specific level M. brevis (5 accessions).

Evaluation:

A common garden study/evaluation plot of select *Gymnocladus dioicus* accessions was established in 2017. This evaluation plot includes 52 wild collected accessions from across the species native range with typically 3 mother trees from each accession, replicated 5 times totaling 720 trees. The main goal is to identify superior accessions of *G. dioicus* germplasm. *G. dioicus* has recently become one of the more popular, widely planted, urban street trees.

We continue to capture observation data on nine *Betula nigra* accessions (145 trees), a common garden study hoping to identify elite lines for the Midwest. Data captured to date includes chlorophyll concentration, caliper, and tree height.

In 2019, we shipped samples of *Salix* (3) and *Quercus* (2) to The National Laboratory for Genetic Resources Preservation in order to better understand optimum storage regimes by testing moisture and temperature variables.

A total of 1309 records were attached in GRIN as either images (634) or as documents (675) including publications, collection trip reports, viability cards, permits and/or passport data.

Enhancement:

A second generation grow out of *Quercus prinoides* (Ames 23752) established in 2016 totaling approximately 150 seedlings continues to be maintained. Seedlings will be screened for mildew resistance and superior growth habit. A hybrid of *Quercus prinoides* x *Quercus macrocarpa* was accessioned as Ames 35261 *Q.* x *beckyae*, which is not known in the nursery trade.

Coordination of the NC7 Regional Ornamental Trials:

In 2019, the horticulture project distributed 17 plants of two accessions to ten sites for long-term evaluation. An additional 3 plants were shipped to three sites. Accessions distributed included *Aesculus glabra* and *Salix eriocephala*.

Posters, Presentations, and Seminars:

In 2019, Jeff Carstens hosted a tour of the NCRPIS to the Iowa State University Horticulture (Plant Propagation) class (38 students); Iowa State University Seed Science Center Seed Analyst Short Course class (10 students); Iowa State University Reiman Gardens Staff (6 individuals); University of Northern Iowa Native Seed Producers/Stakeholders meeting (11 individuals); Story County Iowa Conservation (23 individuals); and a seed threshing demonstration for The American Public Gardens Association (50 participants). Carstens was filmed for an Oklahoma Gardening channel organized by Oklahoma State University highlighting the efforts of the NPGS. Carstens and Andy Schmitz (The Brenton Arboretum) were interviewed for a podcast titled "Mad about Kentucky Coffeetrees" recorded by the University of Minnesota Urban Forestry Outreach, Research & Extension. Carstens co-authored a poster presented at the 2019 American Public Gardens Association conference in hopes to increase awareness of genebanking of plant germplasm.

Conclusions and Plans for 2020:

The 2018 growing season was generally productive in terms of overall regeneration of the horticulture collections. Progress continued in the acquisition and curation of *Monarda* germplasm. Future acquisitions will switch to sampling of western *Fraxinus* species, *Monarda* spp., *Agastache* spp., and collaboration with Kevin Conrad to sample *Cladrastis kentukea*.

Curation:

For 2020, we will attempt to obtain seed increases from 35 horticulture accessions. Significant time will likely be spent reviewing plant inventory and removal of successfully increased accessions. A number of accessions could be assigned PI numbers and paperwork to complete inactivations.

E. Maize Curation (M. Millard, V. Bernau, B. North, D. Zimmerman)

Project Management:

The maize curatorial tech team is fully staffed with the hiring of Dr. Vivian Bernau, a new maize geneticist/curator in March 2019. Curators Mr. Mark Millard and Dr. Vivian Bernau are assisted by full time ISU Agronomy Department staff, David Zimmerman (Agricultural Specialist II), and full time USDA-ARS staff, Brady North (Biological Science Technician).

Acquisitions:

The GEM program in Ames provided seed to distribute four new GEM populations, and seed to regenerate 30 new BGEM inbred lines. The Raleigh GEM team donated four new GEM populations, 14 CIMMYT Maize Lines (CMLs), five Ohio inbred lines, and one Pennsylvania inbred line. The National Lab for Genetic Resources Preservation (NLGRP) provided seed for 19 accessions under Plant Variety Protection expiring in 2020 and 16 finished inbred lines from Holden Foundation Seeds Inc. The Matthew Hufford Lab at Iowa State University provided seed for an Ab10 version of B73 (with Kelly Dawe, University of Georgia) and the new Reference Assembly versions of the Nested Association Mapping (NAM) population parents. One accession of Looney Corn was received from David Shields (University of South Carolina)

In March 2019, Mark Millard was notified by Shawn Kaeppler (University of Wisconsin-Madison) that Bayer was willing to clear LH244 (PI 612589) from PVP protection in order to make a commercially relevant, easily transformable inbred line available to the public scientific community. The PVP office cleared the accession for division from the NLGRP voucher sample for transfer and distribution at the NCRPIS. Since demand was expected immediately after the announcement of its availability, the NCRPIS distributed 10 kernels for each request. There was not sufficient seed for phytosanitary testing. LH244 is expected to be in high demand over the next 30 years (expected distribution life of an accession increase), so a planting four times larger than standard was planted. Additionally, seed was sent to Wisconsin for increase as insurance of a good first year increase. The NCRPIS now has 170,000 seeds for distribution, despite the difficult season.

Due to repeated increase failures, alternate seed sources for inbred lines WD and W438 were received from Shawn Kaeppler (University of Wisconsin-Madison). A good increase was obtained from WD in Ames this summer.

Viability testing determined that seed donated by the University of Minnesota for inbred lines was dead. A number of collaborators were contacted in search of alternate sources of these lines. Lance Veldboom (Bayer Crop Science) provided a new seed source for three Minnesota inbred lines (A25, A297, A635HT), which will be increased in Ames when resources are available.

Two accessions from the Buckler Lab at Cornell and two accessions from the Plant Gene Resource of Canada (PGRC) Genebank were requested for comparison with existing accessions in the collection.

1,200 accessions of tropical maize were transferred from the NLGRP in October 2019 for distribution and management in Ames. The material transferred included about 500 accessions with previous unsuccessful regeneration attempts, and 700+ new accessions. Accessions with distributable amounts are being prioritized for processing and will be assigned PI numbers if viability is good. Critical backup samples of these accessions are being sent back to NLGRP after processing, as possible based on seed inventory.

Collection Maintenance:

There were 20,896 accessions of *Zea* in the active collection at the NCRPIS as of December 31, 2019. The maize curators maintain an additional 53 accessions from the *Coix* and *Tripsacum* genera (Table 1). 373 additional accessions were added in 2019 as inactive accessions until they can be evaluated for incorporation into the active collection based on their viability, quality, and importance to the maize community. (These are discussed more fully under Germplasm Management.)

There were 14,786 available Zea accessions held at the end of 2019 (70.58% of the total). This slight decrease in availability (0.5%) may be largely attributed to the continued identification of deteriorating accessions as maintenance viability testing increased. This is the first time that the number of available accessions has dropped in more than 15 years. Due to a large number of accession inactivations it is also the first time the total number of accessions has dropped. Low viability dictated making 173 accessions unavailable. The harvest and processing of field increases enabled providing new distribution lots of 405 accessions. Progress has not and will not be possible without the in-kind regeneration assistance of private companies, the GEM programs in North Carolina and Iowa, and others.

Table 1. Total number of accessions and available accessions in the NCRPIS maize collection

	Yearly Accession Availability								
Year	Total Accs	Available Accs	Percent Available	New Accs					
2010	20,347	13,338	65.60%	178					
2011	20,540	13,572	66.10%	180					
2012	20,579	13,753	66.80%	39					
2013	20,624	13,757	66.70%	39					
2014	20,694	13,876	67.70%	98					
2015	20,744	14,144	68.20%	53					
2016	20,819	14,144	69.10%	110					
2017	20,952	14,718	70.25%	105					
2018	20,996	14,915	71.04%	33					
2019	20,949	14,786	70.58%	115					

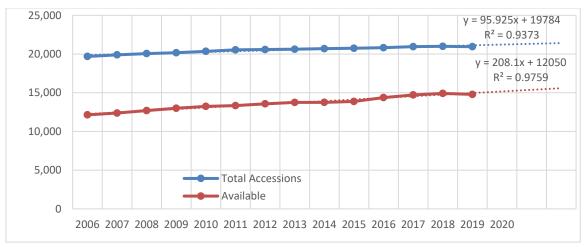


Figure 1. Total number of accessions and available accessions in the NCRPIS maize collection.

Regenerations:

The number of regeneration attempts dropped from 352 accessions (1.7%) in 2018 to 296 accessions (1.4%) in 2019. This compares with 338 regeneration attempts in 2017 (1.6% of the collection), and 442 (2.1%) in 2016.

The hiring of temporary workers in Ames during the summer pollination period continues to be difficult. Notably, Ames, IA was identified as having the lowest unemployment in the nation this summer. As in 2018, a portion of the planned nursery was dropped due to staffing uncertainty. Wet spring weather also delayed and subsequently reduced planting of a significant portion of the Ames nursery. The number of successful attempts was further reduced significantly by poor field conditions during the 2019 growing season. The nursery was composed of 29 expired or expiring PVPs, 30 BGEM inbreds, 54 other inbreds, 14 GEM populations, and 72 other populations. Four planting dates occurred on April 24, May 7, June 3, and June 4 in order to spread out the pollination season. A total of 199 accession regenerations were attempted. Twenty GEM lines were regenerated by the Ames GEM team in 2019 for the maize collection.

The maize curation team increased its first genetically engineered PVP accession. KW7791 (PI 608567) from KWS SAAT AG contains the CRY1Ab gene. In order to achieve a sufficient isolation distance from the other maize regenerations, this accession was planted at the Iowa State University Ag Engineering and Agronomy Research Farm in Boone, Iowa in partnership with ISU collaborators growing an observation field. In order to confirm GEO status, individual plants were tissue-sampled for a GEO ELISA at the Iowa State Seed Science Center. The PVP for this accession expired in February 2020; we await approval for distributing this accession.

No Stewart's wilt was observed in any increase plots in 2018, as in every year between 2010-2018. ELISA testing is still necessary on Ames increase lots to meet phytosanitary requirements because the state cannot be declared Stewart's wilt free.

Ames greenhouse increases included ten inbred accessions planted in February 2019. One population of *Zea luxurians* (teosinte) was planted in June 2019 for a late autumn

harvest. The pots were treated with Paclobutrazol (Bonzi®, Syngenta Crop Protection, LLC; Greensboro, North Carolina) during the growing season to control height and to encourage tillering. In early September they were moved to the greenhouse and placed under LED lights set to short day photoperiods. They almost immediately started shooting tassels, and harvest ran from mid-October through December resulting in. This teosinte is the only accession of the species in the collection.

Quarantine maize was grown in a NCRPIS winter greenhouse for the second time during the winter of 2018-19. During the polar vortex event of 30 January 2019 (in the middle of the federal shutdown), the quarantine greenhouse dropped below 40F. Overnight the hot water line from the university broke and heating to this section of the greenhouse was crippled. Alarms alerted university staff to the problem, and fans were used to move warm air from heated areas toward the quarantine room. Water lines were frozen and broke. Plants next to outer walls were lost but most survived. Increases on the population in the room was successful, but a handful of the inbred lines will need to be grown a third time to get through quarantine. These inbreds had poor tassel emergence and pollen shed. Repairs, including the installation of new benches, began in the fall of 2019 delaying planting five inbred lines from Thailand by a month (until December) for the 2019-20 cycle.

A tropical nursery consisting of 121 mid-altitude to lowland accessions was sent to Semillas Moreno Retis (SMR) in Nayarit, Mexico in the fall of 2018 and harvested in early 2019. Accessions were chosen based on successful increases from the 2017-2018 nursery. Processing was completed in November 2019. Our target population of 100+ears was reached with 48 accessions. More than 60 ears were received for 72 accessions.

We shipped an additional 62 populations for a third increase by SMR in September 2019. This shipment presented many challenges. First, a false positive appeared in a bulk Goss's wilt ELISA test. We retested all the component accessions of that bulk. Then, Mexican authorities would not clear 10 accessions which were originally increased in Mexico between 1986 and 1995 because we did not have an original export permit; these accessions were returned to us. To replace the returned accessions, we shipped 10 other accessions that were grown in the United States.

To review, this nursery was set up to assist in regenerating accessions that would grow best in the tropics. We have seed of several thousand accessions that were grown in Peru, Mexico, and Colombia in the 1986-1995. We will need to work this problem and determine where/how we can increase these non-US produced seed lots.

St. Croix grew 25 accessions in 2019. At present, APHIS will not issue a permit to grow quarantine maize in the field, only in a greenhouse. Dr. Goenaga is working with others in ARS to try to remedy this situation.

CIMMYT grew 25 Peruvian highland accessions in Toluca, Mexico under USDA-ARS contract in 2018. These regenerations, received in March 2019, were very successful. So, 25 additional accessions were sent in March 2019 for a second highland nursery. However, due to delays from the government shutdown and shipping issues, these accessions were planted in March 2020.

Availability and Backup:

One-hundred-fifty-six accessions were backed up at the NLGRP in Fort Collins in 2018. This compares with 202 in 2018, 97 in 2017, 56 in 2016, and 101 in 2015. The percent of the collection backed up held at 74% (15,532 accessions). Currently, NLGRP needs are not the highest priority for regenerating, but are considered. Since there is a large backlog of regenerations needed, viability of the Ames inventory and availability are the more important priority setting factors. At storage of a new increase, NLGRP holdings are reviewed for each accession and seed is sent to NLGRP if their backup is considered substandard.

Viability Testing:

There were 1,681 accessions tested for viability in 2019 (8% of the collection); 1,888 maize accessions tested in 2018 (9% of the collection). Most accessions are tested on a 10-year plan. In 2018 we began doing indicator tests of two reps of 10 seeds on older lots of unavailable accessions with few seeds or accessions that have not been germinated in over 10 years. The idea is to find those accessions that have the poorest viability to determine regeneration priorities and overplanting needs. We plan to continue doing a statistically sound full germination test of four reps of 50 seeds on new increases. It is hoped that the indicator tests will help us catch up on the 10-year cycle. 10,380 available distribution lots (67.0% of available accessions) have tests more than 15 years old. The expected duration for good viability of a maize regeneration in our storage conditions is 30 years. The current distribution lots of more than 25% of the available accessions (and 40% of the whole collection) were grown in 1990 or earlier, so we expect to see more accessions being made unavailable with further maintenance testing (Figure 2).

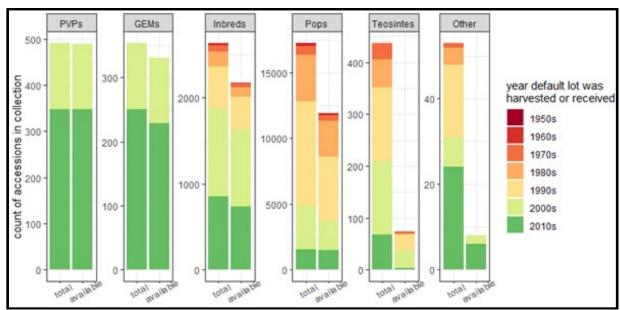


Figure 2. Comparison of available to total number of accessions according to accession type. Each accession is colored according to the approximate age of the current or last distribution lot.

Germplasm Management:

196 accessions were inactivated in 2019. Inactivation of an accession occurs when it has been determined the accession cannot or should not be maintained as part of the

active inventory of the North Central Regional Plant Introduction Station (NCRPIS). These included 22 accessions of maize from the P.C. Mangelsdorf/W.C. Galinat (PCM/WCG) collection. Forty-nine accessions of *Tripsacum* collected by the Natural Resources Conservation Service (NRCS) failed to germinate. Twenty accessions of landraces collected from the Yucatan peninsula and four popcorn accessions were probably received as non-viable seed. An additional 64 accessions were inactivated due to duplication.

209 new PI numbers were assigned in 2019. Preparations for PI number assignment include proofing and updating of all passport data, determining that there are no duplicate accessions, verifying accession viability, and determining whether any Material Transfer Agreement (MTA) or Intellectual Property Rights (IPR) restrictions apply to the material. PI numbers were assigned to 73 North Carolina inbred lines, 73 CMLs, 30 BGEMs, 17 landraces from the northern United States and Canada, six progenitors of the Iowa Stiff Stalk Synthetic (BSSS) and 10 other inbred lines that are parents of the Nested Association Mapping (NAM) population.

With the addition of a second maize collection curator, a backlog of donated material including 771 inventory lots representing 389 accessions were inventoried. Due to unknown viability and utility to the maize community, the majority of these accessions (303 accessions, 572 inventories) were added as inactive, temporary accessions. These accessions are primarily inbreds donated by Major Goodman in the 2000s and popcorn inbreds and populations donated by Ken Ziegler from the Iowa State popcorn breeding program in 2005. Several large seed lot donations remain to be inventoried, including 23 boxes of maize races and 281 packets of inbreds donated by Major Goodman in 2009. The maize staff plans to regenerate and evaluate these accessions for activation as resources are available.

Following a vote at the Maize CGC meeting in December 2019, 1,444 accessions donated as the PCM/WCG collection were inactivated in January 2020 (not reflected in the statistics reported here). The accessions represent maize and wild relatives collected across Latin America by PCM, WCG, and their collaborators between 1930 and 1980, and donated to the NPGS in 1990. Before inactivation, V. Bernau updated the passport information associated with the accessions according to a hand-written catalog that accompanied the collection. When provided in the catalog, collection and donor source information was uploaded for all accessions, and local and collector names were added to the accession records. Though they are no longer part of the active collection, these accessions will continue to be stored at -18°C.

Distributions:

Orders for all accessions that are maintained by the maize curator including those of the genera *Tripsacum* and *Coix* increased 107% in 2018 compared to a 7.2% increase in 2017, a 15.7% increase in 2016, a decrease of 12.4% in 2015. In 2019, distributions returned to a level intermediate between 2018's and 2017's. A good share of the continuing increase was due to four orders over 1,000 accessions. Insect and pathogen resistant screening and phenotyping/genotyping were reasons given. Almost all orders are now entered by requestors in GRIN-Global. Expired PVP-lines continue to be a major maize distribution category followed by NAM inbred parents, the Goodman-Buckler inbred diversity set, and all other inbred lines. Targeted requests for fewer

accessions continue to comprise the bulk of the orders. Handling this number of packets would be very difficult without GRIN-Global and the hard work of Ms. Stacey Estrada, Ms. Lisa Burke and their teams in order processing and seed storage.

Table 2. Yearly distribution statistics for the maize collection

	Annual Distribution Data											
Year	Total Packets	Foreign Packets		Foreign Accs	Orders	Foreign Orders	Requestors	Foreign Requestors				
2015	13,860	3,427	4,444	2,008	528	87	414	80				
2016	13,541	4,252	4,572	2,707	611	121	473	106				
2017	12,541	2,095	4,137	1,042	655	99	451	73				
2018	25,993	10,846	8,833	4,834	671	131	456	107				
2019	17,139	5,025	5,065	2,217	722	126	456	104				
Averages 2015-19	16,615	5,129	5,411	2,562	637	113	450	94				

Orders for expired PVPs were sent to 306 requestors (39% of all *Zea* requestors). Expired PVPs made up some portion of 42% of all *Zea* orders shipped.

Characterization and Evaluation:

Over 1752 images on over 861 accessions were loaded to GRIN in 2019. This increased the maize images on GRIN to over 16,764 images on 7,559 accessions.

There were 22,067 data points on 1,504 accessions loaded to GRIN in 2019. 16,173 data points on 1,241 accessions loaded into GRIN in 2018. This compares with 16,173 data points on 1,504 accessions in 2018, 11,324 data points on 1,279 accessions in 2017, and 9,715 data points on 1,718 accessions loaded in 2016. We imaged 861 accessions in 2019 compared with 565 accessions in 2018, 499 accessions in 2017, and 527 accessions in 2016. There were at least 271 images taken on 189 inactive accessions that were being evaluated for incorporation into the collection or were inactivated in 2019.

NSL and Ames numbered accessions will continue to be reviewed, and PI numbers assigned. As of March 2020, 214 PI numbers have been assigned this year. Over 910 additional Ames-numbered and NSL-numbered accessions need to be reviewed and considered for permanent PI numbers in 2020.

We will continue acquiring germplasm from public collections. We received a portion of Don White's inbred collection from Dr. Tiffany Jamann at the University of Illinois in January.

We will augment the collection of 16,764 images currently on GRIN-Global with images of additional accessions in 2020. Efforts to digitize and edit images for upload will also continue in 2020.

Bayer Crop Science provided in-kind screening of 100 inbreds for Goss's wilt resistance in Nebraska and 55 inbred accessions for tar spot resistance in Illinois. The

data has been received and awaits GRIN-Global loading. This screening is done along with screening of GEM germplasm.

Corteva Agriscience provided in-kind screening of 32 inbreds for fusarium resistance and 55 inbreds for head smut resistance. Again, this screening is done along with screening of GEM germplasm.

Publications and Outreach:

Curator V. Bernau conducted an analysis of various nursery success metrics for all increase nurseries over the last 10 years. Results of this analysis were presented at the ASA/SSSA/CSSA annual meetings in San Antonio, TX in November 2019. Analysis of the historical data revealed that Ames nurseries generally had a significantly higher stand per kernel planted (after thinning) and more pollinations were attempted per plant than nurseries provided by most of our contractors and partners. However, ultimately there were few significant differences between the numbers of ears and kernels produced per kernel planted across all sites. Most importantly, analysis lead to the implementation of modified data collection protocols, including the capturing of additional data points and a full suite of data on accessions planted as checks in each nursery.

Plans for 2020:

In 2020, as in recent years, attending to regenerations and regeneration processing will need to take precedence. Without viable seed, distribution and resulting research cannot be done. To better focus these regenerations, we look forward to updated viability status for outstanding maize populations from the germination team.

When the initial batch of 1,200 accessions of tropical increases initiated by Major Goodman in the 1990's has been processed and stored, we will continue transferring seed from NLGRP at Fort Collins, to Ames for distribution and management. It is easiest for NLGRP to sendout accessions as a set of accessions received together. Shipments with distributable amounts of seed (>2500 kernels) are being prioritized. Transferring the 11,284 packets is expected to take about 10 years. More than 5,000 accessions are expected to require regeneration.

A tropical regeneration nursery in Puerto Vallarta is being planned for 2020.

A highland nursery of 25 accessions was planted in Toluca, Mexico in March 2020.

NSL and Ames numbered accessions will continue to be reviewed, and PI numbers assigned. As of March 2020, 214 PI numbers have been assigned this year. Over 910 additional Ames-numbered and NSL-numbered accessions need to be reviewed and considered for permanent PI numbers in 2020.

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We will augment the collection of 16,764 images associated with 7,559 accessions currently on GRIN-Global with images of additional accessions in 2020. Efforts to digitize and edit images for upload will also continue in 2020.

F. Oilseed Crops (L. Marek, G. Welke, J. Schwartz)

Project Management:

Curator Dr. Laura Marek was assisted by full time Agronomy Department staff, Grace Welke, ISU Agronomy Assistant Scientist III, and by Jeff Schwartz, USDA-ARS Agricultural Research Technician. John Reinhardt, ISU Farm Equipment Operator works with the NCRPIS Oilseed Project November through March. Due to NC7 Hatch Project funding constraints, Mr. Reinhardt worked very limited hours during December 2019. The project is also supported by a team of hourly student workers. Oilseeds regenerations were increased about 20% as a result of having the second full time technician position filled. As Mr. Schwartz gains understanding of the GRIN-Global database, we are also prioritizing work on data entry backlogs and image loading.

Acquisitions:

The oilseed project received seven new accessions in 2019.

Helianthus:

Three cultivated *H. annuus* pre-breeding lines were received from Dr. Brent Hulke, USDA plant breeder, Sunflower and Plant Biology Unit, Fargo, ND.

One wild perennial *H. divaticatus* sunflower accession, collected in West Virginia, was received from Jeff Carstens, USDA horticultural curator, NCRPIS.

Two wild annual sunflower, *H. exilis*, collected near Healdsberg, CA were received from Dr. Tom Gulya, retired USDA plant pathologist.

Flax:

One sample of the wild perennial flax, *Linum lewisii*, was collected in West Virginia by Jeff Carstens, USDA horticultural curator, NCRPIS. *L. lewisii* is commonly found in the western U.S., however, disjunct populations occur in West Virginia. The collection by Mr. Carstens represents the first sample from the West Virginia "Appalachian strain" of Lewis' flax in the NPGS collection.

Collection Maintenance:

General statistics about availability and management of the collections are presented in Appendix Tables 1 and 2. Selected details for oilseed accessions increased during 2019 are noted below.

Helianthus, Ames regenerations:

Cultivated *H. annuus* accessions are 91% available for distribution. We manage regenerations to ensure that core collection accessions and other accession subsets of interest to specific stakeholder groups are available.

In 2019, 105 cultivated *H. annuus* accessions were regenerated in the field including two CMS lines, eight UGA-SAM1 association mapping population lines, 24 Canadian pre-breeding lines and 10 recently released inbred lines from the USDA's Fargo breeding program (Figure 1). Seed was harvested from all plots and processing is under way.



Figure 1. 2019 cultivated sunflower regeneration field, rows and cages.

Wild annual *Helianthus* accessions are 96% available and wild perennial accessions are 83% available. Six new wild annual and 18 new wild perennial sunflower regenerations were started in Ames in 2019. One of the perennial accessions will be inactivated because no original seed remains and plants grown from seed from two increase years were not the correct species. One previously established perennial plot from 2017 was re-caged. Processing of harvested seed is underway.

Typically, two or three oilseed accessions requiring long seasons or short days to flower are increased in an NCRPIS greenhouse during the winter. In winter 2018-2019, we attempted three accessions of wild sunflowers: one did not germinate, one did not flower and one, the annual *H. anomalus*, did very well. For winter of 2019-2020 we attempted two accessions in the miscellaneous asters group, *Centrapalus* ssp. (one did not germinate) and one cultivated sunflower. One accession of the wild annual species *H. debilis* ssp. *debilis* developed for ornamental and dune restoration purposes is maintained clonally in the greenhouse and distributed as vegetative clones.

Miscellaneous asters:

The miscellaneous asters are 35% available. Two *Centrapalus* accessions were attempted for 2019-2020 winter greenhouse regeneration; one did not germinate.

<u>Helianthus</u> and <u>Miscellaneous asters</u>, <u>Parlier alternate grow-out site regenerations</u>: We continue to partner with the National Arid Lands Plant Genetic Resources Unit (NALPGRU), Parlier, CA to regenerate wild taxa requiring a longer growing season

than is reliably obtained in Ames as well as those species that do not grow well in midwestern humidity and heavy soils. The Parlier location uses cages purchased by the NCRPIS and can grow up to 40 NCRPIS oilseed accessions per year. We germinate seeds in Ames and ship live seedlings to Parlier in late March and early April. Parlier staff transplant the seedlings and manage plant growth. Following our Ames protocol, plots are caged before flowering, pollinator insects are introduced (honeybee services supported with Ames resources), and seed heads are harvested as they mature. Harvested material is shipped to Ames for threshing and processing.

In 2019, seedlings of 37 wild sunflower accessions were shipped to Parlier. Two perennial sunflower accessions planted in 2018 were maintained for second harvests in 2019. One miscellaneous aster accession planted in 2018 and which did not flower in the fall, survived the winter was harvested in fall 2019.

The Parlier staff record basic field observations (transplant, flowering and harvest dates and takes some images) but they do not have the resources to record standard descriptor data such as ray and disc flower color, plant height, and branching characteristics nor to take all images. Phenotypic information is a valuable component associated with each accession and it is important to capture the observation data. In September 2019, Ms. Welke and I traveled to Parlier to record descriptor information and to take images.

We have an excellent partnership with the NALPGRU staff, ensuring successful regenerations of many taxa. We are most grateful for the dedicated efforts of Dr. Claire Heinitz, Curator, and Mr. Jerry Serimian, Agricultural Research technician, and their staff.

Brassicaceae regenerations:

Brassicaceae accessions are 86% available. Availability is lower than in past years because viability maintenance testing of distribution lots of *Brassica* accessions (started in 2016) determined a larger than expected decrease in viability (some lots fell by as much as 80% in the six or 10 year interval since prior testing), with 385 lots at 50% or less viability.

The majority of accessions with low germination distribution lots are *B. napus* (83%), most of which are of winter flowering type, requiring a vernalization period to induce flowering. In past attempts, few winter flowering type accessions have survived the winter in the field in Ames; therefore, we start winter flowering types in the greenhouse and vernalize them in a NCRPIS vernalization space (Figure 2). In December 2018, we started 49 *Brassica* accessions, seeds of one did not germinate and 48 accessions were kept in the greenhouse until late February 2019. One accession bolted in early February, information had not been available concerning its flowering type, and that accession was kept and caged in Farm Greenhouse 2 (GH-2) where it flowered, matured and was harvested. The 47 non-bolting accessions were moved to the new NCRPIS vernalization rooms and then transplanted to the field. In addition, forty spring flowering type *Brassica* accessions were planted; 30 were direct seeded into field plots, 10 were started in germination boxes and seedlings were transplanted to the field. Plots were harvested and seed processing is underway. Seventy-five

'probable' winter flowering type Brassica accessions were started in late November 2019 for growth, vernalization and spring 2020 transplanting to the field.



Figure 2. NCRIPS vernalization space with 2019 Brassica ssp regenerations.

One winter hardy *Camelina* accession was planted in fall 2018 in two field locations, both to observe its survival in Ames and, due to steady interest in winter hardy *Camelina*, to increase available inventory. The accession grew very well in one of the fields and that harvest is being processed.

NCRPIS Greenhouse #2 (GH-2) is managed in the winter to provide conditions that approximate a Mediterranean climate allowing us to regenerate Brassicaceae accessions native to that region, and to grow other Brassicaceae taxa which flower very early in the growing season. In fall 2018, we started 11 accessions in GH-2, eight Camelina spp accessions, two Thlaspi arvense accessions and one Lepidium sativum accession. In addition, one Lepidium sativum and one Crambe laevigata, planted in previous years and kept growing due to low seed inventory, were harvested. Two of the Camelina accessions were among a group of nine donated without specific epithet from a 2014 collecting exploration in Armenia. We were able to determine taxonomic clarification and started the remaining seven Camelina spp. in fall 2019 to complete the taxonomic clarification effort. An additional eight Brassicaceae species were started in fall 2019, one each of Biscutella laevigata, Camelina sativa, Camelina rumelica, ssp transcaspica, Crambe kralikii, Erysimum menziesii, Hesperis matronalis, Parrya nucicaulis and Sinapis arvensis ssp arvensis.

Linum regenerations:

Cultivated flax accessions are 99% available; wild flax accessions are 81% available. Ninety-seven percent (2778 accessions) of the large cultivated flax collection was transferred to Ames in 1998/1999 and was of uniform seed age. The NCRPIS viability lab determined that seed viability had started to decline for some distribution lots and increased maintenance germination testing for this crop completing the current viability testing cycle in 2019. Based on the resulting data, we are now regenerating 45 accessions of cultivated flax every year, with priority given to accessions with distribution lots less than 50% viability with the long-term goal that all distribution lots have at least 80% viability. In 2019, 45 cultivated flax accessions with distribution lots with viability ranging from 12 to 82% were regenerated. 2019 maintenance germination testing identified 556 of 1014 tested accessions with viability scores of less than 80%, 206 were less than 60%, and these will be the focus of future regenerations. No wild flax accessions were attempted in 2019.

Cuphea regenerations:

No Cuphea regenerations were attempted in 2019. Seeds are available for 94% of the accessions of seven species (Cuphea calophylla, C. carthegenensis, C. lanceolata, C. lutea, C. tolucana, C. viscosissima, C. wrightii) and for the Cuphea hybrid accessions that were part of agronomic development efforts of the now inactive National Cuphea Consortium. Thirteen accessions of Cuphea are maintained as clones in the greenhouse and distributed as vegetative cuttings. Overall, the Cuphea collection is 80% available.

Euphorbia regenerations:

The *Euphorbia* collection (210 accessions) is 49% available. The taxon of interest for seed oil production within this genus is *E. lagascae* and its accessions are 89% available. Nine *Euphorbia lagascae* accessions were attempted in 2019. Two accessions will be inactivated due because seeds did not germinate. One accession had two seeds germinate but the seedlings did not survive to be transplanted to the field. Seeds were harvested from the six accessions that did grow in the field and are being processed. Six *Euphorbia* accessions are maintained as clones in the greenhouse and distributed as vegetative cuttings.

Distributions:

287 orders containing 14,197 Oilseed packets were shipped in 2019. General statistics about oilseed distributions are presented in Appendix Table 3. A summary of the distributions separated by international versus domestic distributions is presented in Table 1 below.

Table 1: Summary: Oilseed Crops 2019 Distributions

	shipped		international	international	international	domestic	domestic	domestic	total
crop	orders 2019	packets	orders	packets	requestors	orders	packets	requesters	requesters
Helianthus	128	10013	45	6225	35	83	3810	64	100
Brassicaceae	102	3879	25	1837	23	77	2042	66	89
Linum	18	196	6	38	6	12	159	12	18
Cuphea	7	22	3	14	2	4	8	3	5
Euphorbia	8	22	2	3	2	6	19	6	8
misc Asters	14	117	0	0	0	13	118	13	13

Helianthus:

Roughly 56% of the total *Helianthus* items distributed in 2019 (10,013 packets) were sent to seed companies or other commercial entities, 73% of which were sent to international destinations reflecting current locations of the majority of commercial sunflower breeding programs. Programs associated with public institutions, primarily universities, and national institutes received 44% of the distributions. The majority of sunflower packets (64%) were sent to support breeding programs which is the category the majority of the seed companies identified as the research purpose. About 16% of the total distributed items were sent for UGA-SAM1 and GB_UBC prebreeding line evaluations (1632 packets) focused on drought, salt, and flooding tolerance as well as disease resistance. Eight percent of the distributed packets were sent to support plant pathology research. Sixty-one percent of the total distributed packets were of cultivated *H. annuus* accessions, 25% were wild annual sunflowers including wild *H. annuus* and 11% were wild perennial sunflowers.

Brassicaceae:

The genus *Brassica* accounted for 68% of the 2019 Brassicaceae distributions (2626 total packets, 1605 unique accessions). The Brassicaceae genus with the second largest number of packets distributed was *Eruca*: 584 packets, 15% of the total. The diversity present in the Brassicaceae collection (262 taxa in 21 genera) supports a very wide range of research purposes including genetic studies, details of which vary widely, pathology research related to disease resistance, varietal development, bioremediation and investigations of plant-produced defense compounds.

Linum:

Seventy percent of the flax packets distributed in 2019 were wild flax accessions. Eighty-one percent of the distributions were sent to domestic requesters, including the largest single distribution to a research program focused on the domestication of perennial flax. In addition, wild flax accessions were sent to support archaeobotanical and other comparative taxonomic research. Cultivated flax accessions were requested to support entomological and archaeobotanical research.

Cuphea:

Cuphea accessions were distributed in 2019 for research programs studying thioesterases and fatty acids, for anthropological research and for varietal development.

Euphorbia:

Euphorbia accessions were distributed in 2019 for a range of research purposes including anthropological research, plant pathological investigations, weed science and to a program looking at alternative natural rubber sources.

Miscellaneous asters:

As noted in Table 1 above, all the distributions of miscellaneous aster packets were sent to domestic researchers in 2019. Eighty four percent of the packets were sent to a researcher exploring the possibility of a genetic analysis of the genus *Centrapalus*. Other stated research purposes included anthropological research, plant pathology investigations, entomology investigations and distributions to a program looking at alternative natural rubber sources.

Research Activities:

General statistics about observations for the collections are presented in Appendix Table 4.

Helianthus:

SAM evaluations/Pre-breeding lines: We cooperate with the NSF and Genome Canada projects which involve field and greenhouse evaluations using the UGA-SAM1 association mapping population and the GB_UBC developed pre-breeding lines. The projects are managed by Dr. John Burke and Dr. Lisa Donovan, UGA, USA and Dr. Loren Rieseberg, UBC, Canada. We provided seeds for evaluations of floral volatiles and floral traits under drought, for the mapping of hydraulic traits and mapping floret characteristics related to pollinator visitation, Cooperators

laboratories are in the US (Georgia, California, Washington and Florida), Uganda, Chile, India and Canada.

Pre-breeding Lines: Loren Rieseberg's lab at the University of British Columbia developed the GB_UBC pre-breeding lines with support from The Global Crop Diversity Trust. The Crop Trust provided funding for an international evaluation effort of these lines, "Evaluation of Sunflower Pre-Bred Lines for Stress Resistance and Associated Trade-off with Yield", managed by the University of British Columbia group with partners in Chile, Argentina, Africa, India, Israel and Ames. We contribute to the partnership by providing seeds for the international locations and recording descriptor data during the regeneration process. Funding was received in 2017 (\$25,000) for two years with a no-cost extension ensuring all funds are spent by June 30, 2020.

Brassicaceae:

Brassica rapa flowering type evaluation: We are in process of evaluating the 544 accession *B. rapa* collection for flowering type (winter or spring). Knowing whether or not vernalization is required to obtain flowering (winter type), allows more efficient management of regeneration efforts. Spring flowering types can be direct seeded in the field in the spring, a much less resource intensive process than growing seedlings in the greenhouse, transferring to and maintaining plants in a vernalization location, followed by transplanting to the field. In 2018-2019, we evaluated the second set of 80 accessions of which 59% were spring type (flowered fully without vernalization). In November 2019 we started a set of 100 accessions. Observation priority was determined based on viability data; lowest viability accessions were evaluated first so that those accessions could be incorporated into regeneration priorities.

Brassica napus winter survival of winter flowering types: In fall 2018, 15 Brassica napus and two B. rapa winter type accessions previously determined to have some percentage of winter hardiness in Ames (successful vernalization and regeneration by overwintering in the field) were direct seeded in a common garden plot to confirm their winter hardiness using multiple seed lots (seeds from winter surviving plants and original seeds). There is general interest in more winter hardy Brassica both to improve the yield for canola production and to improve the performance of Brassica used as a winter cover crop. Twelve of the 17 accessions had plant regrowth in spring 2019, with survival rates ranging from 20 to 84%. Five accessions had zero to few plants survive. Thirty percent of accessions for which some plants survived in a previous winter did not do well during the 2018-2019 winter in Ames which reinforces the necessity of using our standard regeneration protocol for winter flowering type brassicas, using the greenhouse and vernalization chamber and then transplanting to the field versus direct fall seeding in the field.

Professional Activities:

Meetings and Presentations:

<u>January</u>: I participated in the National Sunflower Association's 41nd Annual Research Forum in Fargo, ND, and led the satellite Sunflower CGC meeting which due to the federal government shutdown, USDA colleagues were not able to attend.

I participated in the Plant and Animal Genome XXIV Conference in San Diego and the one-day satellite meeting of the Genome Canada and NSF sunflower group. I gave a short presentation about regenerations, seed stocks and seed order processes for the UGA-SAM1 association mapping population which both groups are using for trait evaluations. I also participated in a meeting with the Crop Trust's sunflower GB_UBC pre-bred lines evaluation group.

March: Attended the RF Baker Plant Breeding Symposium, ISU.

<u>August</u>: Participated in the NCRPIS RTAC meeting in Urbana, IL and made a short presentation about the status of the Oilseed Project.

<u>September</u>: Attended the 31st annual AAIC meeting in Tucson, AZ and provided a report to the New Crops Crop Germplasm Committee on oilseed crops of interest held at the NCRPIS: Brassicaceae, *Euphorbia*, *Cuphea* and the miscellaneous asters genus *Centrapalus* ssp.

Training:

Throughout the year, I completed safety trainings as required including Epipen, Fire Extinguisher use, and assigned AgLearn modules.

Publications:

Book chapter:

Marek LF. 2019. "Sunflower". In: "North American Crop Wild Relatives, Volume 2. Important Species" SL Greene, Williams KA, Khoury CK, Kantar MB, and Marek LF, editors. Cham Switzerland, Springer, pp. 453-483.

Poster:

Seiler GJ and Marek LF. 2019. Exploration for Coastal Dune Sunflower from the Barrier Islands off the Gulf Coast of Florida. Crop Science Society Meeting, San Antonio, TX, November 12.

Active Grants:

Crop trust:

In 2017 the ISU Curator for the Oilseeds project received a \$25,000 grant from the Global Crop Diversity Trust administered through the University of British Columbia to support regenerations of the Canadian developed GB_UBC sunflower pre-breeding lines received in 2016. The grant also provided partial support of my travel to the project's annual meetings at the Plant and Animal Genome conferences in San Diego, CA in January.

Collecting:

I served as the PI for a USDA Plant Exchange Office funded Plant Exploration Grant for David Brenner to collect wild relatives of amaranth, millets, bedding plants, corn and quinoa in Florida. This grant was closed out at the end of 2019.

Service Activities:

Tours:

- American Public Gardens tour, April 2
- Stacey Harmer, cooperator UC Davis, May 7
- Visitors from NPGS NLGRP, July 23
- Agronomy 522 class tour, July 24
- Agronomy Predictive Plant Phenomics tour, August 22
- Visiting graduate student from Nebraska, sunflower, August 22

Journal peer review:

I served as a peer reviewer for submissions to Crop Science and Scientific Reports.

Book editor:

I served as one of five editors for a two-volume book about crop wild relatives being published by Springer. The final volume, North American Crop Wild Relatives, Volume 2. Important Species, was published in January 2019.

CSSA Genetic Resources C08:

Frank Meyer Medal committee member

Grant Panel Reviewer:

I served as a reviewer for the NIFA SAC panel during June and July; teleconference virtual meeting of the panel was held in July.

<u>Plant Germplasm Operating Committee (PGOC)</u>:

I serve as a member of the PGOC GIS and Geo-referencing Subcommittee and the Molecular Subcommittee.

G. Vegetables (K. Reitsma, L. Clark, C. Hopkins)

Collections curated by the Vegetable Project include *Cichorium* (NC7-chicory), *Cucumis sativus* (NC7-cucumis.cucs), *Cucumis melo* (NC7-cucumis.melo), *Cucumis* species (NC7-cucumis.wilds), *Cucurbita pepo* (NC7-cucurbita), *Daucus* (NC7-daucus), *Ocimum* (NC7-ocimum), and *Pastinaca* (NC7-parsnips). Statistics for accession numbers and availability for each site crop are found in the appendices in "Table 1: NCRPIS Accessions (Accs), Acquired, Available", but are also summarized specifically for the Vegetable Project in the table below.

									Percent	
	Number			Percent	PI	Ames	NSL		Backed	
Number	Accs	Number	Percent	Avail Last	Numbered	Numbered	Numbered		up	
Accs	Acquired	Available	Available	Year	Accs	Accs	Accs	Backup	NCGRP	Svalbard
285	1	244	86	86	230	28	26	258	91	114
1401	3	1335	95	95	1230	143	25	1331	95	985
3224	8	1946	60	61	2906	279	27	2597	81	551
318	0	208	65	64	245	73	0	208	68	51
979	0	732	75	74	884	90	5	824	84	298
1563	82	1226	78	82	968	486	30	1322	89	940
106	0	99	93	93	93	13	0	100	94	76
73	0	58	79	79	52	19	2	58	68	33
7949	94	5848	77	74	6608	1131	115	6698	84	3048
	285 1401 3224 318 979 1563 106	Number Accs Acquired 285 1 1401 3 3224 8 318 0 979 0 1563 82 106 0 73 0	Number Accs Accs Number Available 285 1 244 1401 3 1335 3224 8 1946 318 0 208 979 0 732 1563 82 1226 106 0 99 73 0 58	Number Accs Accs Available Number Available Percent Available 285 1 244 86 1401 3 1335 95 3224 8 1946 60 318 0 208 65 979 0 732 75 1563 82 1226 78 106 0 99 93 73 0 58 79	Number Accs Accylined Number Available Percent Available Available Year 285 1 244 86 86 1401 3 1335 95 95 3224 8 1946 60 61 318 0 208 65 64 979 0 732 75 74 1563 82 1226 78 82 106 0 99 93 93 73 0 58 79 79	Number Accs Accs Acquired Number Available Percent Available Available Available Available Available Available Accs Numbered Accs 285 1 244 86 86 230 1401 3 1335 95 95 1230 3224 8 1946 60 61 2906 318 0 208 65 64 245 979 0 732 75 74 884 1563 82 1226 78 82 968 106 0 99 93 93 93 73 0 58 79 79 52	Number Accs Acquired Acquired Number Acquired Percent Available Available Year Numbered Accs Numbered Accs Numbered Accs 285 1 244 86 86 230 28 1401 3 1335 95 95 1230 143 3224 8 1946 60 61 2906 279 318 0 208 65 64 245 73 979 0 732 75 74 884 90 1563 82 1226 78 82 968 486 106 0 99 93 93 93 13 73 0 58 79 79 52 19	Number Accs Accs Number Available Percent Available Available Available Numbered Accs Ac	Number Accs Number Accs Number Acquired Percent Available Percent Avail Last Year Percent Accs Numbered Accs <	Number Accs Number Accs Number Accs Number Accs Available Available Available Available Available Available Numbered Accs Accs

Acquisitions:

One expired PVP of *Cichorium endivia* from Nunhems Netherlands, BV was received from NLGRP. Three *Cucumis sativus* and eight *Cucumis melo* cultivars from NLGRP were received for incorporation into the NPGS collection due to interest from cooperators. Eighty-two *Daucus* accessions received via the Global Crop Diversity Trust Crop Wild Relative initiative included germplasm from Armenia (5), Azerbaijan (5), Cyprus (8), Georgia (3), Italy (29), Nepal (1), Pakistan (13), and Portugal (18). The collection included five accessions each of *Daucus carota* subsp. *azoricus* and *Daucus carota* subsp. *halophilus*, two new taxa for the NPGS collection.

Collection Maintenance:

Data for vegetable crop regenerations attempted and number of accessions harvested in 2019 are summarized in the appendices in "Table 2: NCRPIS Accessions (Accs) Germinated, Regenerated, Made Available, Backed Up."

Availability of the Cucumis sativus collection is at 95% with only 65 accessions unavailable for distribution. Most of the unavailable accessions require specialized regeneration protocols and greenhouse regeneration. Cucumis sativus field cage regenerations focused primarily on accessions with distribution seed lots twenty years or older, with lower seed quantities and lower viabilities. Of the 96 accessions regenerated, 28 had distribution seed lots between 20 and 28 years old, and 56 were between 30 and 40 years old. (Cucumis seeds tend to retain high viability for 20 to 30 years or longer.) Fourteen of the accessions had viabilities below 80% or seed quantities below 1500 ct. The remaining twelve accessions regenerated had low seed quantities. The 2019 seed increases are still being processed and will be viability tested in April 2020. Regenerations were attempted on 16 accessions of wild Cucumis species in the greenhouse with nine producing moderate to good quantities of fruit/seed, one accession failed to flower (possibly due to day-length), and four accessions produced low quantities of fruit/seed. No Cucumis melo accessions were regenerated at the NCRPIS in 2019. Melons will not be regenerated in significant numbers until a reliable protocol is developed to deal with the seed transmitted bacterial fruit blotch (Acidovorax citrulli) disease.

Cucurbita pepo field regenerations focused on accessions with low seed quantities or distribution lots 20-plus years old. Thirty-seven accessions were planted for field cage regeneration and three accessions for fall greenhouse regeneration. Seeds of eight accessions failed to germinate, three accessions had poor germination resulting in insufficient plant populations for regeneration, and plants of one accession were destroyed due to high levels of seed transmitted squash mosaic virus (determined through ELISA testing of all cucurbit seedlings prior to transplanting to cages). Twenty-three accessions were transplanted to field cages. Preliminary assessment of harvested seed indicates 14 accessions with good to excellent, seven with moderate, and five with low seed quantities. Moderate to good seed quantities were produced on the three greenhouse regenerations. Accessions that failed to germinate will be grown from alternative inventory lots for regeneration in 2020. Accessions that produced low fruit quantities in field cages may be grown again in 2020 either in field cages or the greenhouse. Seeds of the accession found positive for squash mosaic virus will be

dry heat treated prior to planting in 2020 to try to eradicate the virus. All 2019 increase lots will be inventoried and stored after viability testing in April 2020.

The NCRPIS *Daucus* regenerations included 11 biennials and 56 annuals. Accessions were grown to replace old distribution seed lots and seed lots with low seed quantities. Also included were 20 new wild species accessions from Tunisia, Spain, and the United States. Cleaning of the 2017 *Daucus* increases was completed and seed lots have been viability tested, inventoried and stored resulting in 78 inventory lots being made available for distribution. Threshing and blowing has been completed on the 2018 *Daucus* increases with about 50% of the accessions having been picked and sampled for germination testing. About 95% of the 2019 harvests have been threshed. In addition to the Ames regenerations, we received substantial seed increases of six cultivated biennial accessions each from Laura Maupin, Seminis Vegetable Seeds (Monsanto), Idaho and Rob Maxwell, Bejo Seeds, Idaho.

Seven accessions of *Pastinaca* with distribution seed lots between 22 and 28 years old and declining viability were regenerated in field cages. Good harvests were made on six of the seven accessions. One accession had a small plant population and will be regenerated again in 2020 and the resulting increases will be bulked together.

As NCRPIS accessions are regenerated, backup seed samples are sent to the NLGRP in Ft. Collins. Backup samples of 60 accessions from the Vegetable Project were sent to the NLGRP in 2019. Overall, 84% of the accessions in the vegetable collections are backed up. Six of eight vegetable site-crops have 80% or more of their accessions backed up at NLGRP (Appendix Table 2).

Distributions:

Packet and accession distributions for research and education for the vegetable collections are summarized in the appendices in "Table 3A: External NCRPIS Distributions". In 2019, 9368 items (packets) involving 4283 accessions were distributed to fulfill 199 orders (121 domestic, 78 foreign) equaling 171 unique recipients. Vegetable research requests received in 2019 specified objective topics as disease evaluations, breeding for specific traits and disease resistances, genetic and molecular studies, and diversity assessment for biotic and abiotic stress tolerance. The set of 691 Daucus accessions was distributed four times in 2019 to participants in the Carrot SCRI (Specialty Crops Research Initiative) coordinated by Dr. Philipp Simon (USDA-ARS, Madison, WI). Seed companies continue to request substantial numbers of accessions primarily from the cucurbit collections, but all available Pastinaca accessions were sent to one international seed company – a collection that doesn't typically receive much interest from other than home gardeners. International requests for approximately 4760 items from the NCRPIS Vegetable Project collections were cancelled for 15 international orders because we did not receive import permits, were unable to satisfy import requirements, or the requestors were not able to secure waivers for the additional declarations on the import permits.

Non-Research Requests (NRR) continue to make up a significant portion of the cancelled Vegetable Project requests.

Germinations:

In 2019, 733 vegetable inventory lots were tested for viability (Appendix Table 2), with the majority of the testing attributed to maintenance germinations of *Cucurbita pepo* and new increase lots.

Characterization and Taxonomy:

Digital images and basic notes for taxonomic identification and accession characterization were recorded during regeneration. Data for approximately 17 descriptors (primarily fruit descriptors) were recorded at harvest for *Cucumis* and *Cucurbita*. Plant habit, flowering dates, and life-cycle notes were recorded for *Daucus*. Images taken of vegetable accessions in 2019 will be loaded to GRIN-Global. These data and images will be made available via GRIN-Global.

Taxonomic identities are reviewed and confirmed as each accession is regenerated. No taxonomic changes were submitted in 2019, but several are pending for *Cucumis* and *Daucus*.

Evaluation/Utilization:

We continue to screen all *Cucurbita* and *Cucumis* seedlings grown for regeneration for the presence of squash mosaic virus, using ELISA, before seedlings are transplanted to the field cages. All cucurbit field and greenhouse plantings are also visually inspected for disease during the growing season. Seed-borne diseases are of specific interest, with bacterial fruit blotch (*Acidovorax citrulli*) in *Cucumis melo* being of particular concern. Phytosanitary issues have prevented the distribution of *Cucumis* and *Cucurbita* germplasm to some countries. The Vegetable Project is working with Dr. Anna Testen and Dr. Narinder Pal (NCRPIS Pathology Project) to develop a method to clean up infested seed lots and prevent transmission of seed borne diseases such as bacterial fruit blotch. The goal is to develop a seed treatment to eliminate the pathogen during the regeneration process which will enable us to secure disease-free seed lots for distribution. Please refer to the Plant Pathology section of this NCRPIS annual report for more details.

David Spooner (USDA, ARS, Madison, WI) is wrapping up work on the taxonomic revision of the genus *Daucus* and allied species as he prepares to retire in the Fall of 2020. There are still pending publications as well as labeling and disposition of herbarium specimens collected during the project. The specimens will be deposited in the University of Wisconsin herbarium in Madison.

The Daucus SCRI Project (initiated in 2017) lead by Dr. Philipp Simon (USDA-ARS) has a goal of using applied genomics to develop enhanced Daucus carota breeding lines based on evaluations of the NPGS cultivated Daucus carota accessions for nematode resistance, heat tolerance, flavor and diversity analysis, stand establishment, cavity spot evaluation, and bolting. A set of 690 NCRPIS accessions of cultivated wild germplasm has been distributed as nine separate orders to participants in the SCRI since 2017. The set is being evaluated in Wisconsin and California fields for flowering time, stand establishment, top size, nematode and Alternaria resistance, flavor, root nutritional pigments, cavity spot, and root appearance (shape, external and internal color). Images of typical roots are also being collected.

The SCRI and the Crop Trust projects are developing breeding pools by intercrossing plants with superior performance for traits mentioned above. They are also genotyping phenotyped plants for both projects and developing a database.

The CucCAP Project (started in 2016) had three objectives: develop genomic approaches and tools for cucurbit species, perform genomic-assisted breeding to introgress disease resistance into cucurbit cultivars, and perform economic impact analyses of cost of production and disease control and provide readily accessible information to facilitate disease control. NPGS crop specific curators participated in the project providing information and guidance with regard to the germplasm collections and the NPGS. For NCRPIS germplasm collections, CucCAP evaluations focused on disease resistance in *Cucumis sativus* (downy mildew, Phytophthora), Cucumis melo (powdery mildew, Fusarium, Cucumber Yellow Stunting Disorder Virus, Cucumber Mosaic Virus), and Cucurbita pepo (powdery mildew, Phytophthora, Papaya Ring Spot Virus, Cucumber Mosaic Virus). All genotype by sequencing (GBS) was completed for cucumber, melon and watermelon by April 2018. The GBS for the Cucurbita collections were completed and the data made publicly available via the CucCAP website, though the data has not yet been published. Phenotypic characterization of the cores was also planned but may have to be pursued under the The project's website, https://cuccap.org/, posts a list of proposed CucCAP II. publications resulting from the research, and provides access to cucurbit genomics tools and databases via the Cucurbit Genomics Database website. All phenotypic data generated in the evaluation process will be referenced in or made available via the GRIN-Global database, and enhanced lines developed through the process may be made available through the NPGS.

Publications/Posters:

C.K. Khoury, D. Carver, H.R. Kates, H.A. Achicanoy, M. van Zonneveld, E. Thomas, C. Heinitz, R. Jarret, J. Labate, K. Reitsma, G.P. Nabhan, S.L. Greene. 2019. Distributions, conservation status, and abiotic stress tolerance of wild cucurbits (Cucurbita L.). Plants People Planet (PPP). doi: 10.1002/9993.10085.

F. Martínez-Flores, M.B. Crespo, P.W. Simon, H. Ruess, K. Reitsma, E. Geoffriau, C. Allender, N. Mezghani, and D.M. Spooner. Subspecies Variation of *Daucus carota* coastal ("gummifer") Morphotypes (Apiaceae) Using Genotyping-by-Sequencing. Final revision submitted to Systematic Botany 01/09/2020.

Plans for 2020:

Regenerations:

Six *Daucus setifolius* and 28 *D. crinitus* accessions from Morocco, Portugal, and Spain were planted in the greenhouse in early November 2019 for regeneration in 2020. Three of the *D. crinitus* failed to germinate but we will try them again in 2021. Little information is available regarding regeneration requirements for these perennial species, so this will be a learning process as we progress through the seed increases. We may also direct seed 20 to 30 annual *Daucus* accessions into field cages in late spring of 2020 depending upon the progress of the perennial increases. The plants are growing very slowly and may need to remain in the greenhouse for regeneration. We will regenerate approximately 100 *Cucumis* in field cages and again focus on

accessions with distribution lots thirty years or older, low seed quantities, or low seed viabilities. Approximately 30 *Cucurbita pepo* accessions will be planted for field cage regenerations. Regenerations of wild *Cucumis* species and hard-to-handle *Cucumis* and *Cucurbita* will continue in the greenhouse as time, space, and resources permit.

The Vegetable Project will continue to collaborate with the Plant Pathology project researching new regeneration protocols for the cucurbit collections to produce pathogen free seed lots, primarily regarding the *Acidovorax citrulli* pathogen in the *Cucumis melo* collection.

Characterization:

We still have many years of fruit characterization data on cucurbits to be converted and loaded into GRIN-Global. Image file naming continues in preparation for mass loading digital images acquired since 2013 on seeds, plants, and fruits. Naming and image loading protocols are still being developed in coordination with the NCRPIS Imaging Committee. A student employee is also scanning "historical" *Cucumis melo* photographs for loading to GRIN-Global as it may be many years before an accession is regenerated and a new digital image of an accession can be acquired. Review of accession passport data will continue for the cucurbit and *Daucus* collections in preparation for assigning PI numbers to many of the Ames-numbered accessions in the collections (414 *Cucumis*, 88 *Cucurbita*, and 99 *Daucus*).

Evaluation:

We are awaiting receipt of evaluation and characterization data resulting from the NPGS funded proposal "Phenotypic and molecular marker evaluation of carrot and wild *Daucus carota* germplasm recently added to the NPGS" submitted by Drs. Philipp Simon and David Spooner (USDA-ARS, Madison, WI) through the Root and Bulb Vegetable Crop Germplasm Committee (RBV-CGC) in 2014. Phenotypic evaluation for key carrot descriptors (storage root shape and color, annual - biennial flowering behavior, other RBV-CGC approved descriptors), and Alternaria leaf blight susceptibility will be collected on the 167 wild and domesticated carrot germplasm accessions collected for the NPGS from 2007 to 2013. Genotyping-by-sequencing (GBS) will be used to characterize the genetic diversity of the germplasm. These data will be integrated with other genomic data to study carrot genetics, domestication, speciation, and evolution. All phenotypic data collected will be loaded into GRIN-Global.

Data generated by the CucCAP (Project Director: Dr. Rebecca Grumet, Professor, Dept. of Horticulture, Michigan State Univ., East Lansing, MI) and the *Daucus* SCRI (Project Director: Dr. Philipp Simon, USDA-ARS, Vegetable Crops Research Unit, Madison, WI) will be loaded to GRIN-Global with the completion of the projects.

H. Research Leader Activities (C. Gardner)

Administration and Leadership Activities:

Gardner administers the five-year project plan objectives for the USDA-ARS Plant Introduction Research Unit's two CRIS Projects, Plant Introduction Research and the

Germplasm Enhancement of Maize (GEM) Project and contributes to the coordination and execution of activities which support those objectives. Gardner serves as the Coordinator of the Hatch-funded Multistate NC7 Project. New five-year project plans for the NC7 Project and both of the ARS CRIS projects were approved in 2017.

Budgetary anomalies due to changing Congressional and Agency priorities continue to command more time and resources. Because of delays in release of funds to the management unit, each year we deal with uncertainty. Making timely decisions for work plans for many taxa that require germination and vernalization treatments in the winter is challenging under these circumstances. The GEM Project CRIS continues to be leveraged to support maize curatorial activities as well, not a permanent solution.

Gardner serves on the advisory group for the Daucus SCRI Project. She served on the selection panel for a new soybean Cat 4 geneticist/curator for the Urbana soybean genebank in 2020, following service on a previous search for this position in 2019.

About 10% of her time was devoted to assisting GRIN-Global System development team members, 15% to the GEM Project, 5% to activities related to implementation of the new optical sorting technology, and about 70% to genebank issues and writing in the past year.

The VMEK Metrix is a fast, visual optical sorter than has enabled us to optimize maize seedlots that will result in much better emergence and remove contaminants from a variety of crop seeds. Heavily used by the GEM Project to optimize seedlots for yield trials planting with air planters, some Agronomy Department faculty have also used it to sort and optimize seedlots of soybean, millet, and sorghum with good success. We will be experimenting with a variety of other crop seeds to determine if we can eliminate much manual seed picking prior to storage.

Posters were presented about use of our optical seed sorters at the Assoc. for Industrial Crops meeting in Arizona in September 2019, and also at the Crop Science Meetings in Texas in November 2019.

2020-2021 Plans:

The VMEK Metrix optical sorter will continue to be used to experiment with sorting 'recipes' for a number of crops to explore quality improvement of seed lots. It offers a lot of promise.

The QSorter from the Swiss company, QualySense, purchased with USDA-ARS Midwest Area and HQ support, captures 3D images and NIR spectra from seeds, and can sort seeds based on calibrations developed for specific traits or size/color parameters. QualySense has enabled the instrument research-read and we will now focus on collecting image and NIRS data in preparation for algorithm development activity. The viral pandemic delayed progress in this area, as the company plans to locate a research and development team in Ames in the near future.

Pete Cyr will continue to focus on development of RESTFUL interface applications to enable ready extraction of GRIN-Global information that can be combined with information from other resource providers (such as genomic information resources) by researchers.

We continue to focus on recruiting to fill vacant PIRU and NCRPIS positions with outstanding individuals, facilitate smooth transitions, and assist graduate students in completion and publication of their work. We will continue to use the ORISE program to hire contract employees to cover some aspects of our activities. Once the new horticultural research technician starts in June 2020, we will have no vacant positions since 2014 – hopefully for a long time except for pending retirements. Gardner will enter phased retirement (50%) in July 2020, and a new Research Leader will be recruited for the Unit. It has been an honor and privilege to work with everyone at the NCRPIS, the NPGS, Iowa State University, the NC7 RTAC members and their institutions, and our Academic Advisors in ISU's College of Agriculture and Life Sciences.



Year 2019 01/01/2019 t		N(CRPIS Acc	essions (A	ccs), Acqui	red, Availa	able
	GENUS_CROP	Number Accs	Number Accs Acquired	Percent Acquired	Number Available	Percent Available	Percent Avail Last Year
Brenner	NC7-grass.echinochloa	315	2	1%	284	90%	89%
	NC7-grass.misc	142	9	6%	83	58%	59%
	NC7-grass.panicum	936	0	0%	902	96%	97%
	NC7-grass.setaria	1115	4	0%	1016	91%	91%
	Subtotal Grasses:	2508	15	1%	2285	91%	91%
	NC7-legume.melilotus	1006	0	0%	899	89%	89%
	NC7-legume.misc	299	0	0%	160	54%	53%
	Subtotal Legumes:	1305	0	0%	1059	81%	81%
	NC7-pseudocereal.amaranth	3337	1	0%	3232	97%	97%
	NC7-pseudocereal.celosia	60	1	2%	39	65%	64%
	NC7-pseudocereal.perilla	25	0	0%	24	96%	88%
	NC7-pseudocereal.portulaca	13	1	8%	10	77%	
	NC7-pseudocereal.quinoa	451	3	1%	283	63%	
	Subtotal Pseudocereals:	3886	6	0%	3588	92%	93%
	NC7-spinach	413	0	0%	314	76%	85%
	NC7-umbels	1196	6	1%		63%	63%
	Brenner Total:	9308	27	0%	8005	86%	87%
Carstens	NC7-medicinals	1084	31	3%		71%	
	NC7-ornamentals	767	29	4%	528	69%	
	NC7-woody.landscape	2028	64	3%	1058	52%	54%
	Carstens Total:	3879	124	3%	2356	61%	63%
Marek	NC7-asters	450	8	2%		35%	
1,1011 011	NC7-brassica	2019	0	0%	1690	84%	
	NC7-crucifers	1307	24	2%	1164	89%	
	NC7-cuphea	638	0	0%		80%	
	NC7-euphorbia	210	0	0%		49%	
	NC7-flax	2834	0	0%		99%	
	NC7-flax.wilds	167	7	4%		74%	
	NC7-sun.cults	2611	3	0%		92%	
	NC7-sun.wilds.ann	1692	15	1%	1605	95%	
	NC7-sun.wilds.per	899	2	0%	742	83%	84%
	NC7-sun.wilds.sp	2	0				
	Subtotal Wild Sunflower:	2593	17			91%	92%
	Marek Total:	12829	76	1%	11308	88%	84%
Bernau	NC7-maize.coix&tripsacum	53		2%		15%	
& Millard	NC7-maize.gems	354	38	11%		94%	
	NC7-maize.gems	2684	57	2%		80%	
	NC7-maize.pop	16981	0	0%			
	NC7-maize.pvp	491	19	4%		100%	
	NC7-maize.teosinte	439		0%		17%	
	Subtotal Zea:	20949	114	1%		71%	
	Bernau & Millard Total:	21002	115	1%	14794	70%	71%
Reitsma	NC7-chicory	285		0%			
LUCIUSIIIA	NC7-cucumis.cucs	1401	4	0%		95%	
	NC7-cucumis.melo	3224	8	0%		60%	
	NC7-cucumis.wilds	318		0%		65%	
	NC7-cucurbita	979		0%		75%	
	NC7-daucus	1563		5%		78%	
	NC7-ocimum	106	0	0%		93%	
	NC7-parsnips	73	0	0%		79%	
		7949	95	1%	1,0,000	79%	
NCRPIS To	Reitsma Total:	54967	437	1%		74%	

01/01/2019 to	12/31/2019				NUMPIS	Accession	s (Accs) Ge	rminated, Re	generateu	, Made Ava		Number	Number		
CURATOR	GENUS_CROP	Number Accs	Accs	Percent Accs Germed	Number Attempted Regen	Number Harvested Regen	Number Perm Perennial	Number Perennial Harvested (Vegetative)	Number Accs Growing	Number Accs Made Available	Accs Backed	Accs Backed	Accs Backed Up	Total Number Accs Backed Up	Percent Accs Backed Up
Brenner	NC7-grass.echinochloa	315	13	4%	19	1	0	0	0	9	7	0	0	279	89%
	NC7-grass.misc	142	59	42%	8	2	0	0	0	15	6	0	0	94	66%
	NC7-grass.panicum	936	160	17%	8	5	0	0	0	0	0	0	0	916	98%
	NC7-grass.setaria	1115	264	24%	41	41	0	0	0	82	79	0	0	978	88%
	Subtotal Grasses:	2508	496	20%	76	49	0	0	0	106	92	0	0	2267	90%
	NC7-legume.melilotus	1006	8	1%	0	0	0	0	0	8	6	0	0	931	93%
	NC7-legume.misc	299	3	1%	0	0	0	0	0	3	2	0	0	218	73%
	Subtotal Legumes:	1305	11	1%	0	0	0	0	0	11	8	0	0	1149	88%
	NC7-pseudocereal.amaranth	3337	29		97	44	0	0	0	28	20	0	0		
	NC7-pseudocereal.celosia	60						0	0		1	0			
	NC7-pseudocereal.perilla	25	4					0			3				
	NC7-pseudocereal.portulaca	13	1					0			1				
	NC7-pseudocereal.quinoa	451	84	19%		36		0							
	Subtotal Pseudocereals:	3886	120		143	82		0							
	NC7-spinach	413	25			1					0				
	NC7-spinach NC7-umbels	1196	35			60									
	Brenner Total:	9308	687	7%		192		0			134				
Carstens	NC7-medicinals	1084	41	4%						The state of the s					
Carstens														1000000	
	NC7-ornamentals	767	16												
	NC7-woody.landscape	2028	22				9 0,000	18		200					
	Carstens Total:	3879	79	2%		80		29	1000	17.00	7.000			4	
Marek	NC7-asters	450	8												
	NC7-brassica	2019	638					0							
	NC7-crucifers	1307	24	2%				0			0				
	NC7-cuphea	638	67	11%				0			0				
	NC7-euphorbia	210	0					0							
	NC7-flax	2834	1017	36%		45		0			0				
	NC7-flax.wilds	167	15	9%	0	1	0	0	0	6	0	0	0	141	84%
	NC7-sun.cults	2611	532	20%	102	103	0	0	0	99	251	0	0	1837	70%
	NC7-sun.wilds.ann	1692	251	15%	20	2	0	0	0	14	0	0	0	1598	94%
	NC7-sun.wilds.per	899	33	4%	34	1	0	0	0	7	0	0	0	751	84%
	NC7-sun.wilds.sp	2	0	0%	0	0	0	0	0	0	0	0	0	0	0%
	Subtotal Wild Sunflower:	2593	284	11%	54	3	0	0	0	21	0	0	0	2349	91%
	Marek Total:	12829	2585	20%	255	257	0	0	0	170	251	0	0	11185	87%
Bernau	NC7-maize.coix&tripsacum	53	2					0		20.5.3.000	2				
& Millard	NC7-maize.gems	354	44	12%		43					5				
	NC7-maize.inb	2684	680	25%		76					127			4	
	NC7-maize.pop	16981	803	5%				0			16				
	NC7-maize.pvp	491	149	30%	29	28		0			6				
	NC7-maize.teosinte	439	3			2									
	Subtotal Zea:	20949	1679	8%	734	367		0			154				
	Bernau & Millard Total:	21002	1681	8%	734	367		0			154				
Reitsma	NC7-chicory	285	11			0			10.0						
nensina									_				_		
	NC7-cucumis.cucs	1401	96												
	NC7-cucumis.melo	3224	4												
	NC7-cucumis.wilds	318													
	NC7-cucurbita	979		36%											
	NC7-daucus	1563		10%											
	NC7-ocimum	106													
	NC7-parsnips	73		51% 9%											
	Reitsma Total:	7949	707						0	86		0	0	6715	

Year 2019 1/01/2019 to		External	$\textbf{External NCRPIS Distributions} \cdot \textbf{Includes both DI (research and education)}, \ \textbf{RP (Repatriation)}, \ \textbf{OB (Observation)}, \ \textbf{and NR (home garden)} \ \textbf{order}$											
1/01/2015 00	12/51/2015		Exte	rnal Domes	tic Distribu	itions		Foreign Di	stributions	i	External D	omestic and	l Foreign D	istributions
CURATOR	GENUS CROP	Number Accs in Collection	Number Items	Number Accs	Number Orders	Number Recipients	Number Items	Number Accs	Number Orders	Number Recipients	Number Items	Number Accs	Number Orders	Number Recipients
Brenner	NC7-grass.echinochloa	315	41	27	9	9	26	24	5	3	67	46	14	1
	NC7-grass.misc	142	17	16	6	6	1	1	1	. 1	18	16	7	
	NC7-grass.panicum	936	927	906	11	11	4	3	3	3	931	906	14	1
	NC7-grass.setaria	1115	76	60	22	16	921	833		6	997	852	28	
	Subtotal Grasses:	2508	1061	1009	36	30	952	861	13	10	2013	1820	49	40
	NC7-legume.melilotus	1006	55	54	8	8	315	289	5	5	370	331	13	
	NC7-legume.misc	299	13	13	5		2	1	2			14	7	
	Subtotal Legumes:	1305	68	67	11		317	290	7	6		345	18	1'
	NC7-pseudocereal.amaranth	3337	3164	2819	39	33	594	555	16	14	3758	2879	55	47
	NC7-pseudocereal.celosia	60	4	4	3		3	3				7	4	
	NC7-pseudocereal.perilla	25	4	4	3	77.00	0	0				4	3	
	NC7-pseudocereal.portulaca	13	14	9	4	4	1	1	1	. 1	15	9	5	
	NC7-pseudocereal.quinoa	451	463	183	35		796	275	15			280	50	
	Subtotal Pseudocereals:	3886	3649	3019	76	151150	1394	834	28			3179	104	
	NC7-spinach	413	2616	347	18		353	353	1			353	19	
	NC7-umbels	1196	731	209	19		113	112	5			261	24	
	Brenner Total:	9308	8125	4651	141	100,000	3129	2450	52			5958	193	
Carstens	NC7-medicinals	1084	270	190	49	2/14/74/9091	134	123		1,231(9)		293	53	
	NC7-ornamentals	767	61	53	20		2	2				55	22	
	NC7-woody.landscape	2028	179	128	70		27	27	2			154	72	
	Carstens Total:	3879	510	371	115		163	152	8			502	123	
Marek	NC7-asters	450	118	112	14	15.000	0	0			100000000	112	14	
	NC7-brassica	2019	1410	1130	44		1750	1531	14			1632	58	
	NC7-crucifers	1307	729	446	39		529	493	17			551	56	
	NC7-cuphea	638	9	8	5		14	14	3			22	8	
	NC7-euphorbia	210	18	16	5		3	3				17	7	
	NC7-flax	2834	37	35	7		68	68				95	11	
	NC7-flax.wilds	167	122	96	6		16	15				96	9	
	NC7-sun.cults	2611	1844	1011	48		4509	2494	36			2523	84	
	NC7-sun.wilds.ann	1692	871	739	42		3501	1612				1627	68	
	NC7-sun.wilds.per	899	1070	762	23	100000000000000000000000000000000000000	808	746				763	32	
	NC7-sun.wilds.sp	2	0	0	0		0	0				0	0	
	Subtotal Wild Sunflower:	2593	1941	1501	56	10.00	4309	2358	28			2390	84	
	Marek Total:	12829	6228	4355	175		11198	6976	79			7438	254	
Bernau	NC7-maize.coix&tripsacum	53	9	5	8		0	0	18000	1 0000		5	8	
& Millard	NC7-maize.gems	354	993	300	49	1,000	489	298				308	61	
u	NC7-maize.inb	2684	4355	1218	243		1669	977	69			1591	312	
	NC7-maize.pop	16981	3305	2347	214		609	453				2606	256	
	NC7-maize.pvp	491	3280	469	246		2558	489	68			489	314	
	NC7-maize.pvp	439	172	75	50		47	30	14			75	64	
	Subtotal Zea:	20949	12105	4409	562		5372	2247	126			5069	688	
	Bernau & Millard Total:	21002	12114	4414	568		5372	2247	126			5074	694	
Reitsma	NC7-chicory	285	12	12	4		81	81	1	0.000		89	5	
2001001110	NC7-cucumis.cucs	1401	411	367	23		637	410		-		648	44	
	NC7-cucumis.melo	3224	677	590	36		949	735	22			1052	58	
	NC7-cucumis.wilds	318	26	23	4		223	165				168	13	
	NC7-cucurbita	979	507	285	21		109	72				317	29	
	NC7-daucus	1563	3477	921	22		166	143	5			925	27	
	NC7-ocimum	106	55	43	7		4	4	2			44	9	
	NC7-parsnips	73	4	43	4		55	55	1			56	5	
	Reitsma Total:	7949	5169	2245	99		2224	1665	50			3299	149	
The second second second	otal:	54967	32146	16036	1000		22086	13490	296		A CONTRACTOR OF THE PARTY OF TH	22271	1296	

01/01/2019 to	Table 4.				NC	RPIS Acces	ssions (Acc	s) Observation	ns (Obs) in GR	IN, Image	es in GRIN			
	GENUS_CROP	Number Accs in Collection	Number of Accs Obs Trials		with Obs in GRIN	GRIN	Number of Accs with Obs In GRIN Last Year		Number of Accs with Obs in GRIN (all years)	Number of Accs Imaged	Number of Accs with Images in GRIN for Year	Number of Images in GRIN for Year	Number of Accs With Images in GRIN (all years)	Number of Images in GRIN (all years)
Brenner	NC7-grass.echinochloa	315	1	4	1	0	0	1167	305	0	0	0	64	130
	NC7-grass.misc	142	8	9	9	0	0	290	113	1	14	35	30	58
	NC7-grass.panicum	936	2	2723	936	0	0	4256	936	0	0	0	125	240
	NC7-grass.setaria	1115	0		154	1	1	4668			2	2	157	
	Subtotal Grasses:	2508	11	3318	1100	1	1	10381	2432	1	16	37	376	739
	NC7-legume.melilotus	1006	0	250	50	0	0	7485	996	0	58	65	213	309
	NC7-legume.misc	299	0	0	0	0	0	547	244	0	8	16	34	57
	Subtotal Legumes:	1305	0			0				0			247	
	NC7-pseudocereal.amaranth	3337			74	5891	446			37			1210	
	NC7-pseudocereal.celosia	60				0								
	NC7-pseudocereal.perilla	25				0								
	NC7-pseudocereal.portulaca	13				0								
	NC7-pseudocereal.quinoa	451	6		10.00	196								
	Subtotal Pseudocereals:	3886			106	6087							1400	
	NC7-spinach	413				2		8039			047			
	NC7-umbels	1196			1	0					Ü		206	
	Brenner Total:	9308			1257	6090		89135		38			2246	
Carstens	NC7-medicinals	1084				20							U. (1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	
Carstens	NC7-medicinals	767	0			0				0				
		2028	9										885	
	NC7-woody.landscape Carstens Total:		-							0			1538	
Manala	NC7-asters	450								0				
Marek														
	NC7-brassica	2019	82			0					87		419	
	NC7-crucifers	1307	4			0		7325			0			
	NC7-cuphea	638	0			0		4260			v	U		
	NC7-euphorbia	210				0								
	NC7-flax	2834	0	U	0	0					0	U	1	
	NC7-flax.wilds	167	0			0					0			
	NC7-sun.cults	2611	0	U		0		104316			1	-	255	
	NC7-sun.wilds.ann	1692												
	NC7-sun.wilds.per	899				0								
	NC7-sun.wilds.sp	2												
	Subtotal Wild Sunflower:	2593	0			0		00000		0	1		189	
	Marek Total:	12829	100000000						A CONTRACTOR OF THE PARTY OF TH	0		A 100 MARCH 100	THE RESERVE OF THE PERSON NAMED IN COLUMN 1	
Bernau	NC7-maize.coix&tripsacum	53				0								
& Millard	NC7-maize.gems	354	50										165	
	NC7-maize.inb	2684	46		702	3842							645	
	NC7-maize.pop	16981	38		479	9119							6200	
	NC7-maize.pvp	491	153	4640	251	2336	426	22624	510	30	60	402	435	3155
	NC7-maize.teosinte	439	0	0	0	0	0	216	94	1	0	0	107	115
	Subtotal Zea:	20949	287	22067	1505	16173	1241	307121	16597	859	1752	3966	7552	16757
	Bernau & Millard Total:	21002	287	22067	1505	16173	1241	307121	16597	861	1752	3966	7559	16764
Reitsma	NC7-chicory	285	0	0	0	0	0	4700	279	0	0	0	257	913
	NC7-cucumis.cucs	1401	4	0	0	0	0	26149	1377	90	0	0	920	1231
	NC7-cucumis.melo	3224	35	0	0	0	0	12286	3196	0	0	0	649	1074
	NC7-cucumis.wilds	318	0		0	0		680			5	8	75	
	NC7-cucurbita	979			0	0							150	
	NC7-daucus	1563	16		0	0						0	681	
	NC7-ocimum	106				0					0		13	
	NC7-parsnips	73	1	0	0	0				7	Ö			1
	Reitsma Total:	7949	56			0				206		-	2746	6833
Maria de la companya della companya	otal:	54967	581			22426							15297	

Appendix Figure 1

